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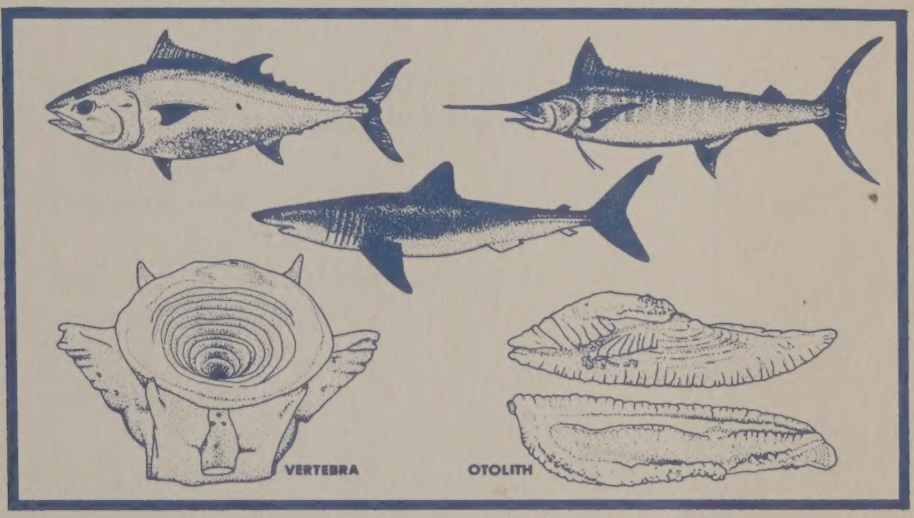
NOAA Technical Report NMFS 8

Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks



Southeast Fisheries Center, Miami Laboratory
National Marine Fisheries Service, NOAA
Miami, Florida
February 15-18, 1982

Eric D. Prince (Convener and Editor)
Lynn M. Pulos (Editor)

December 1983

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

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Malcolm Baldrige, Secretary
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John V. Byrne, Administrator
National Marine Fisheries Service
William G. Gordon, Assistant Administrator for Fisheries

PREFACE

Accurate and precise estimates of age and growth rates are essential parameters in understanding the population dynamics of fishes. Some of the more sophisticated stock assessment models, such as virtual population analysis, require age and growth information to partition catch data by age. Stock assessment efforts by regulatory agencies are usually directed at specific fisheries which are being heavily exploited and are suspected of being overfished. Interest in stock assessment of some of the oceanic pelagic fishes (tunas, billfishes, and sharks) has developed only over the last decade, during which exploitation has increased steadily in response to increases in worldwide demand for these resources.

Traditionally, estimating the age of fishes has been done by enumerating growth bands on skeletal hardparts, through length frequency analysis, tag and recapture studies, and raising fish in enclosures. However, problems related to determining the age of some of the oceanic pelagic fishes are unique compared with other species. For example, sampling is difficult for these large, highly mobile fishes because of their size, extensive distributions throughout the world's oceans, and for some, such as the marlins, infrequent catches. In addition, movements of oceanic pelagic fishes often transect temperate as well as tropical oceans, making interpretation of growth bands on skeletal hardparts more difficult than with more sedentary temperate species. Many oceanic pelagics are also long-lived, attaining ages in excess of 30 yr, and more often than not, their life cycles do not lend themselves easily to artificial propagation and culture. These factors contribute to the difficulty of determining ages and are generally characteristic of this group—the tunas, billfishes, and sharks. Accordingly, the rapidly growing international concern in managing oceanic pelagic fishes, as well as unique difficulties in ageing these species, prompted us to hold this workshop.

Our two major objectives for this workshop are to: 1) Encourage the interchange of ideas on this subject, and 2) establish the "state of the art." A total of 65 scientists from 10 states in the continental United States and Hawaii, three provinces in Canada, France, Republic of Senegal, Spain, Mexico, Ivory Coast, and New South Wales (Australia) attended the workshop held at the Southeast Fisheries Center, Miami, Fla., 15-18 February 1982.

Our first objective, encouraging the interchange of ideas, is well illustrated in the summaries of the Round Table Discussions and in the Glossary, which defines terms used in this volume. The majority of the workshop participants agreed that the lack of validation of age estimates and the means to accomplish the same are serious problems preventing advancements in assessing the age and growth of fishes, particularly oceanic pelagics. The alternatives relating to the validation problem were exhaustively reviewed during the Round Table Discussions and are a major highlight of this workshop. How well we accomplished our second objective, to establish the "state of the art" on age determination of oceanic pelagic fishes, will probably best be judged on the basis of these proceedings and whether future research efforts are directed at the problem areas we have identified.

In order to produce high-quality papers, workshop participants served as referees for the manuscripts published in this volume. Several papers given orally at the workshop, and included in these proceedings, were summarized from full-length manuscripts, which have been submitted to or published in other scientific outlets—these papers are designated as SUMMARY PAPERS. In addition, the SUMMARY PAPER designation was also assigned to workshop papers that represented very preliminary or initial stages of research, cursory progress reports, papers that were data shy, or provide only brief reviews on general topics. Bilingual abstracts were included for all papers that required translation.

We gratefully acknowledge the support of everyone involved in this workshop. Funding was provided by the Southeast Fisheries Center, and Jack C. Javech did the scientific illustrations appearing on the cover, between major sections, and in the Glossary.

Eric D. Prince, Workshop Covener and Editor
Lynn M. Pulos, Editor

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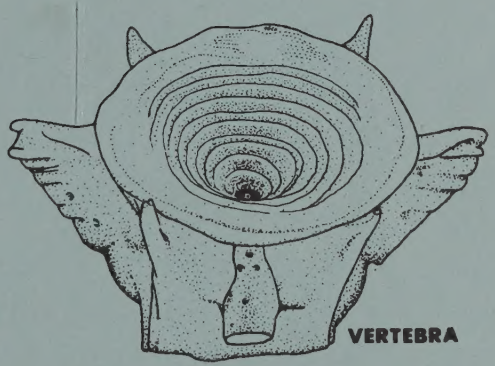
GENERAL OVERVIEWS AND ROUND TABLE DISCUSSIONS



Age and Growth Assessment of Fish from Their Calcified Structures—Techniques and Tools[1]

JOHN M. CASSELMAN[2]

ABSTRACT

Age and growth assessment of fishes from their calcified structures has been used widely for many years, and forms the basis of most of our present-day fisheries management decisions. However, the results of these assessments have not been validated adequately, even in the confines of freshwater, let alone in the oceanic pelagic environment. Five major categories of endeavor should be pursued to improve and refine this science and its practical application: *Interpretation, validation, collaboration, automation,* and *innovation*. Examples of the techniques and tools associated with these approaches are presented in this overview, usually for freshwater species; however, they can be, or have been, applied equally well to oceanic pelagic fishes.

Interpretation of age and growth assessment can be refined and improved by using fluorochrome labels, which provide marks in the calcified structure that permit temporal and spatial orientation. A universally acceptable terminology is needed. Validation of age and growth studies should become routine. Fluorescent markers and tag-recapture are the most useful; however, comparisons of different calcified structures, and other, more indirect tests such as fitting growth models (e.g., von Bertalanffy) can be helpful. Collaboration through exchange programs can produce "reliably" aged reference material now that technology exists to facilitate the transfer of this science. Automation and mechanization of routine age and growth assessment are required. The physical and chemical properties of fish calcified tissue, as revealed by electron microprobe X-ray analysis, substantiate that, as in forestry X-ray densitometry, this approach can be used to mechanize and computerize age and growth assessment of fish. Innovation is necessary to develop new and more powerful techniques that can be used to determine age accurately and precisely. Otolith microstructure has greatly increased precision, and new biochemical (e.g., aspartic acid racemization analysis) and radiometric (e.g., analysis of uranium decay series nuclides ^{226}Ra and ^{210}Pb) techniques have the potential to make age determination truly objective.

INTRODUCTION

Age assessment of fish from their calcified structures is a vital component of most of our present-day fisheries management decisions. Even though this knowledge is used widely, validation of the accuracy of the estimates frequently has been relegated to low priority and often has not even been attempted. Validation should be an essential and routinely performed part of every study that involves the extraction of data from the calcified structures of fish. Although this critical problem is universal (Carlander 1982; Beamish and McFarlane 1983), it has not been adequately addressed even in the confines of freshwater, let alone in the oceanic environment. Probably one of the greatest challenges in validation of age and growth assessment is presented by the large oceanic pelagics such as tunas, billfishes, and sharks. These species are difficult to sample, highly mobile, and have extensive geographic ranges, often encompassing tropical as well as temperate oceans. Nevertheless, the basic principles of age and growth assessment of fish are similar regardless of species and environment, and the practical problems of assessing age and growth of large oceanic pelagic fishes are generally similar to those of other species.

Although comprehensive tests of the reliability of interpretations are few, they indicate that the complexity of the problem has been oversimplified. Some procedures previously considered to be reliable, especially those involving the scale method, are now suspect and under certain conditions have led us to erroneous assumptions. These inconsistencies have affected the confidence that can be placed on this important component of fisheries science. Increased effort is needed to refine, improve, and validate all aspects of the science and technology of age and growth assessment of fish.

If we are to address this problem thoroughly, we must start by considering some very basic problems. For example, inconsistent and ambiguous terminology persists, making communication and comparison of results difficult. This has hindered our ability to transfer science, to better understand the problems, and to develop universal theories explaining the factors causing check and zone formation.

The forum provided by international workshops and symposia, such as the one reported in these proceedings, helps to focus attention and coordinate efforts to resolve such universal problems as those associated with terminology and validation (Brothers 1983). Other international workshops and symposia held in recent years to examine the problem of age determination of fishes (Zoological Society of Slovakia 1968; Bagenal 1974; Everson 1980) not only contributed to better communications and understanding of the problem, but also stimulated additional research. For example, since the Reading symposium in England (Bagenal 1974), studies of otolith microstructure have contributed greatly to the precision with which age can be assessed.

The techniques and tools available to tackle this very fundamental fisheries problem are becoming more numerous, powerful, and sophisticated. Age and growth assessment is undergoing a technological revolution, as are many other fields

[1]Contribution No. 82-04 of the Ontario Ministry of Natural Resources.

[2]Ontario Ministry of Natural Resources, Research Station, Fisheries Branch, Box 50, Maple, Ontario, Canada L0J 1E0.

of scientific endeavor. Fisheries workers must be innovative and apply these modern techniques and tools more widely.

To improve and refine this science and its practical application, five major categories of endeavor should be considered: Interpretation, validation, collaboration, automation, and innovation. Each of these categories will be reviewed with examples of the available techniques and tools. Although many of the examples provided are for freshwater species, they apply equally well to oceanic species, especially the large pelagics, as indicated by examples from this workshop.

INTERPRETATION

Ambiguous terminology has created confusion for those interpreting age and growth of fish. Sometimes results have appeared to be paradoxical when compared with those from other studies and structures. Inadequately defined, ambiguous terminology hinders our ability to transfer information and to develop universally applicable hypotheses. Where possible, we should communicate through a standard terminology (some standardization has been achieved in these proceedings—see Glossary), and if this is not available, then each term should be thoroughly defined.

Terms should describe conditions directly, not circumstantially. For example, optically different zones in fish calcified tissue other than scales should be described according to their structural appearance or light properties, e.g., translucent or opaque, not as "slow-growth-zones" and "fast-growth-zones" or "winter zones" and "summer zones," terms that assume that tissue with a certain optical appearance is deposited in association with slow or rapid growth or particular seasons. The interpretation of results has also been complicated by such ambiguous terms as "light and dark" or "black and white" when the method of illumination is not specified. The terms translucent and opaque should be used, because in the definitions of these terms the type of illumination is implicit—it is transmitted. Translucent means the tissue allows the transmission or passage of light, whereas opaque means that it does not allow the transmission of light, or is impervious to light rays. These terms are preferred and can be used regardless of the method of illumination, because even in reflected light the transmission and absorption of light energy are important.

If it is necessary to describe zonation in reflected light, then the opaque zone does not transmit light energy but reflects it, so the zone appears white or the color of the illuminating light. In reflected light, this zone would be referred to as a reflective zone. In reflected light, the translucent zone allows light to penetrate and be absorbed by the tissue or to pass through the tissue and be absorbed into the background, hence this zone appears darker. This zone would be referred to as an absorptive zone (Casselman 1974).

The term hyaline is acceptable, but has several disadvantages. Although it indicates that the type of tissue is glasslike, vitreous, or free of inclusions, it also means clear or transparent. Calcified tissue is not transparent, but is translucent. Also, the term hyaline has no direct opposite that can be used to describe the "opaque" condition, a term frequently used in juxtaposition. Hyaline explains the nature of the material, whereas opaque explains its light properties.

Terms such as ring, band, mark, and circuli (when not referring to scales) should not be used unless they are adequately described. For example, when talking about ring formation, it is impossible to know the properties (light, structural, or otherwise) of the zone being described. When referring to fish scales, I prefer the term check, which means a break or change in the uniform configurations of the circuli.

Not only is some of the terminology in use ambiguous, but in some cases it is also, by definition, incorrect. For example, the term annulus simply means concentric ring. There is no connotation of yearly in the Latin definition of annulus and it should therefore not be confused with the term "annular." However, the term annulus has become a common and accepted term in age assessment. For purposes of age assessment, the annulus (annual mark, year mark) can be defined as a mark that is subjectively located, sometimes very precisely for "back calculation," on or in a calcified structure; is associated with the distal edge of a concentric ring in the form of a check on the scale or a translucent zone in other calcified structures; is found along the entire structure; and is considered to separate the check or zone associated with the principal annual cessation or reduction in growth from the tissue deposited when growth resumes or increases. Two successive annuli are usually considered to demarcate one calendar year of calcified tissue growth.

Age assessment of fish from their calcified tissue is conducted by systematically interpreting (usually the optical appearance) either a whole or sectioned structure, starting at the focus or origin and examining all regions outwards to the edge. The structure may be treated and examined by different techniques. Nevertheless, the interpretation involves an examination of various checks and translucent zones in terms of their continuity or extent, location, and the quality of the tissue in and about them. The significance of these checks and zones is then judged according to criteria that are based on the definition of the annulus. The checks and zones associated with annuli are differentiated from those considered to be formed at other times and influenced by other factors. Generally, the checks and zones associated with annuli are those that are found throughout the structure and are separated by zones (usually more opaque) associated with growth. The growth zones between annuli usually have characteristics that indicate rapid growth followed by decreasing growth. When specific types of checks and zones are known to be associated with annuli, then the assessment is more objective.

Pseudoannuli, or false annuli, are similar to annuli, but are associated with checks and zones that are somewhat incomplete and irregular, are found in only one part of the structure, and often not in all structures. Although they are sometimes prominent, they are not associated with the check or zone that forms during the "principal annual cessation or reduction" in growth that produces the annulus.

Applying these interpretations results in an age that should be considered to be estimated, assumed, assigned, or assessed. Rarely are the criteria for distinguishing the various types of checks and zones sufficiently precise, or are the techniques adequately validated or even verified so that it can be said beyond reasonable doubt that we have "determined" age. Determination of "true" (correct) age without errors by these techniques from the calcified structures of all fish will probably always elude us. We must recognize and accept the limitations of the method. Age assessment as currently practiced is strongly

subjective. Interpretation can, however, be greatly improved and refined.

When interpreting calcified tissue, it is essential that the examination and description of checks and zones be thorough, and that this information be recorded so it can be evaluated according to objective criteria to obtain age. All too often, age assessment is just a simple enumeration. Regardless of how regular and distinct the checks and zones appear, they should be interpreted in terms of well-defined criteria. Unfortunately, these criteria have not been adequately developed for most structures and species.

If the checks and zones associated with annuli are indistinct, variable in appearance, or coalesce (most frequently at the edge) as a result of decreased growth rate with increased age, then the assessment will be difficult, repeatability will be poor, and results will be inconsistent. Under these conditions the problem should be acknowledged, and the interpretation should be qualified by ranking the degree of confidence. For example, one system provides the number of annuli, a coded description of the edge of the structure (from Casselman 1978, Appendix J), and a numerical ranking (from 1 to 10) of the degree of confidence that can be placed in the assessment. All too often in the past, interpreters accepted the responsibility of providing an age estimate regardless of the difficulty and without indicating any measure of the degree of confidence they placed in the assessment. Unfortunately, even though interpreters examined structures and their images in considerable detail, often nothing more than an estimate of age was provided for further analysis.

An interpretation of a structure provides an estimated or assessed osseological age; however, this may not be the chronological or calendar age of the fish. Since calendar age is required for most fisheries work, it is essential that the relationship between the osseological age and the calendar age be known. If they are the same, the interpretation is valid; if not, the osseological age must be qualified, corrected, or rejected.

In order for an interpretation to be objective and unbiased, no information should be used when the initial interpretation is conducted. It should be made independent of time of capture, length of the fish, and even size of the structure, if possible. The first annulus should not be located by size or, for scales, by number of circuli. This procedure forces all the results to conform to some preconceived interpretation that may not apply to the sample being examined.

Errors in age interpretation undoubtedly occur and, within limits, are acceptable; however, random error is not as important as systematic error (Powers 1983). Serious systematic errors can develop when scales or other structures of fish that are very slow growing, or do not grow, no longer continue to grow and to record age according to normally recognized criteria. Under these conditions, the method results in fish being underaged. This problem is more common than has generally been thought. Indeed, some species may be much older than has heretofore been considered (Beamish 1979). Similarly, certain parts of a structure may continue to grow and indicate age, whereas in other parts, growth may be reduced to a level at which checks and zones are not delineated annually, hence do not represent the actual calendar age.

Specific methods of interpreting age from the various calcified structures have been reported in detail in the literature. However, this overview will refer to only those works selected to describe the various methods or considered important to age determination of oceanic pelagics.

The scale method has been used widely in fisheries, and involves a systematic interpretation of the checks (breaks or changes) in the configurations of the circuli located on the outer surface of the scale (Regier 1962; Carlander 1974; Casselman 1978). The scales are easily removed and magnified either as whole scales or, if thick, as their cellulose acetate impressions. Interpretations have appeared to be straightforward and in some cases even simple; however, when replication is attempted, results are often inconsistent, and verification studies indicate that in older and slow-growing fish, scales underestimate the true age and may be unreliable (Beamish and Harvey 1969; Erickson 1979; Mills and Beamish 1980). A thorough test of the validity of the scale method will demonstrate that in some cases the bias can be great and the method misleading. The scale method has been used frequently for the tunas (Yabuta et al. 1960; Bell 1962a, b; Yukinawa and Yabuta 1963, 1967; Yang et al. 1969; Yukinawa 1970) and Fourier series analysis has been used to determine the time of annulus formation (Nose et al. 1955). The scales of tuna appear to present many of the same problems in age assessment as do those of other fishes (Yabuta and Yukinawa 1963) and are not suitable for ageing billfishes or sharks. Regardless of species, the scale method should be treated with caution and should be avoided if the fish are suspected to be very slow growing or old, because the method will not provide comparable age assessments across a broad range of ages.

The otolith (sagittal) method, which can involve interpretation of either macro- or microzonation, has been used extensively in marine fisheries because workers have recognized that the fish were old and age could be assessed more easily by this method. It is now being applied more widely in freshwater fisheries. The otolith method (macrozonation) involves the recognition and interpretation of translucent zones, which are associated with annuli (although some prefer to enumerate opaque zones). Sagittae can be examined whole or can be fractured, ground and polished, sectioned, stained, charred, acid etched, or otherwise prepared for examination (Blacker 1974). Their removal, preparation, and examination are more complicated and sometimes more difficult than for other methods and also necessitate killing the fish. Nevertheless, the results for many species are more reliable because the interpretations more closely approximate "true" age than those obtained by other methods, especially for old fish (Beamish 1979; Erickson[3]). Results from the otolith method are generally more consistent because zonation is usually more distinct and more easily recognized, even in older fish, than with some other methods. However, this may not be the case for the giant (old) Atlantic bluefin tuna, *Thunnus thynnus*, because estimated vertebral age appears more accurate than does estimated otolith age (Lee et al. 1983).

Detailed microstructure (microzonation) exists in the otoliths of many species, making them especially powerful tools that can reveal even the daily age of the fish (Pannella 1974, 1980; Brothers et al. 1976). The otolith method has been applied to age assessment of tunas (Uchiyama and Struhsaker

[3]Erickson, C. M. 1982. Age determination of Manitoba walleyes using otoliths, dorsal spines, and scales. Manuscr. submitted to North Am. J. Fish. Manage. Department of Natural Resources, Fisheries Branch, Biological Services Section, Winnipeg, Manitoba, Canada R3H 0W9.

1981) and has been examined by using mark-recapture techniques and tetracycline labels to place a temporal and spatial orientation mark on the otolith (Wild and Foreman 1980). The otoliths of the sailfish, *Istiophorus platypterus*, as well as of other istiophorids, contain not only internal zonation, but also external ridges that appear to correspond to age, at least in young fish (Radtke and Dean 1981; Radtke 1983). The otolith method has been applied extensively to the large oceanic pelagic fishes in the present proceedings (Brothers et al. 1983; Hurley and Iles 1983; Lee et al. 1983; Radtke 1983; Wilson and Dean 1983). If fish are suspected to be old, this method appears more useful (see exception, Lee et al. 1983) and zonation should be interpreted along the region of maximum growth, or longest radius.

The fin ray (soft ray) or spine (spiny ray) methods are similar, and offer several advantages over otoliths and other bony structures. These structures can be removed easily, and it is not always necessary to kill the fish or significantly mutilate the carcass (Beamish 1981). The method is especially useful because, like scales, fins can be removed from the fish at time of tagging and compared with the corresponding structure removed at time of recapture. The rays are usually thin-sectioned near the base (Batts 1972; Jolley 1974; Beamish 1981) or the cut surface can be smoothed and illuminated indirectly to expose internal zonation (Deelder and Willemse 1973). Surface examination of whole spines has been used successfully to assess age of spiny dogfish, *Squalus acanthias* (Ketchen 1975). Although rays are useful, there are disadvantages. In older fish the core can undergo resorption and become vascularized, obscuring and even eliminating the first few zones. This would result in an underestimation of age. In old fish, fin rays in some ways are similar to scales because, like checks on the edge of scales, the distal translucent zones may be so close together that they appear to coalesce, making optical resolution and correct age assessment difficult or even impossible. This method is now being used more widely on many species, including oceanic pelagics, as illustrated by its wide application in the present workshop (Antoine et al. 1983; Berkeley and Houde 1983; Cayré and Diouf 1983; Compeán-Jimenez and Bard 1983; Gonzáles-Garcés and Fariña-Perez 1983; Johnson 1983).

The centrum (vertebral) method has not been used widely, although it is an important technique for age assessment of cartilaginous fishes such as rays and sharks (Stevens 1975; Thorson and Lacy 1982) and has been used for several species of tunas. The removal and preparation of vertebrae are more difficult and time-consuming than some of the other methods reported here, and necessitate killing and mutilating the fish. The method involves the surface examination of whole or sectioned centra. The centrum may be viewed in white light, cleared (e.g., cedarwood oil), stained (e.g., alizarine, silver nitrate, or treated in numerous other ways to enhance zonation and facilitate its interpretation (Galtsoff 1952; Cailliet, Martin, Kusher, Wolf, and Welden 1983). This method is examined in detail in this workshop (Cailliet, Martin, Kusher, Wolf, and Welden 1983; Cailliet, Martin, Harvey, Kusher, and Welden 1983; Johnson 1983; Lee et al. 1983; Schwartz 1983) and has been validated with known age (Lee et al. 1983) and partly known age material by using the location of "tagging marks" (Casey et al. 1983) and tetracycline labels (Holden and Vince 1973; Gruber and Stout 1983).

The flat bone method involves either a microscopic, or most frequently a macroscopic, examination of the optical zonation in large, relatively flat bones. This method does not usually involve sectioning or grinding, although the latter may be used to increase light transmission. Fluorescent light enhances optical zonation better than does incandescent light. Incident light with the bone viewed against a dark background appears to be better than transmitted light. The method has been relatively widely used in freshwater fisheries, and has many of the advantages of the other methods (Casselman 1979), although it necessitates killing the fish. The method is especially useful for growth estimation (Casselman 1978). Many types of bones have been used in this method, although opercula (Le Cren 1947; Frost and Kipling 1959), cleithra (Casselman 1974, 1978), and branchiostegals (Bulkley 1960) are probably the most useful. The use of these structures in age assessment of oceanic pelagics has not been adequately documented, although Prince[4] reported that the opercula of the marlins show no conspicuous optical zonation.

Regardless of the method used, age and growth assessment of fish from calcified structures involves an interpretation of growth recorded in the tissue. It is necessary to recognize growth reductions and cessations associated with annual major stoppages that occur at the same time each year, and to distinguish them from those that occur at other times and for other reasons.

Detailed studies that attempt to decode the complete chemical, physical, and physiological record of growth and environmental change in skeletal material of aquatic organisms, as described in Rhoads and Lutz (1980), are rare but have been attempted on some fish, e.g., northern pike, *Esox lucius* (Casselman 1978), and are proposed for others, e.g., lemon shark, *Negaprion brevirostris* (Gruber and Stout 1983). Correct interpretation of this osseological record depends upon a thorough understanding of the factors and physiological processes that influence its growth and check and zone formation. When initially building expertise and acquiring reference information for the accurate interpretation of calcified tissue, it is necessary to understand the environmental requirements of the species.

Temperature is one of the most important factors influencing growth, and the optimum temperature for maximum somatic growth is probably the most useful single value. Although this value varies with species, it can be estimated easily if the final preferendum is determined (McCauley and Casselman 1981). Other major factors affecting growth, such as feeding rate and reproductive cycle, are more difficult to measure, and can be elucidated only by detailed studies in the laboratory and natural environment.

When studying growth in relation to check and zone formation, it is necessary to study the seasonal growth cycle. This is best done in the natural environment and on indigenous fish for which growth history is known. Hence, it is necessary to use mark-recapture techniques and to place temporal and spatial orientation marks (labels) in the calcified structures. Such studies not only provide the scientific basis for age and growth assessment, but also provide reference material that can be used to improve subsequent interpretations.

[4]Prince, E. D., Fishery Research Biologist, Southeast Fisheries Center Miami Laboratory, National Marine Fisheries Service, NOAA, 75 Virginia Beach Drive, Miami, FL 33149, pers. commun. 1982.

Although many types of chemicals have been used to label calcified tissue, fluorochrome labels using an antibiotic such as tetracycline appear to be the best, and have the added advantage of being therapeutic and prophylactic. These fluorochromes are deposited at all sites of calcification, and are visible as a fluorescent band when exposed to ultraviolet light. In studies on northern pike in the natural environment, Casselman (1978) tested intraperitoneal, subcutaneous, and intermuscular injections. An injectable solution of oxytetracycline hydrochloride, which contained 100 mg/ml Liquamycin,[5] marked scales and bones best when the fish were injected intraperitoneally with dosage rates of 25 to 50 mg/kg body weight. However, the type of injection and dosage rate depend upon many factors, including growth rate, type of tissue being marked, type of mark desired, and required longevity.

Tetracycline has been used to elucidate the seasonal growth cycle of the calcified tissue and body of northern pike (Casselman 1978). Shown in Figure 1 are the seasonal dynamics of the qualitative growth of cleithra and scales, and the quantitative linear growth of the body, cleithra, and scales of northern pike tagged and recaptured throughout the year in a small, shallow lake. Checks and translucent zones associated with annuli (tissue type 1) were deposited during late winter and early spring (Fig. 1B). Widely spaced circuli and opaque cleithral tissue (tissue type 4) were deposited during early and midsummer. Most rapid cleithral and scale growth occurred during early summer (Fig. 1D), coinciding with the optimum temperature for growth (Casselman 1978). These data substantiate that the annulus formed on the scales and in the cleithra at approximately the same time, when growth was slowest, and only once each year. Maximum and minimum growth rates of both structures and the body coincided seasonally. During rapid growth, the scales grew linearly at a faster rate than did the bones, and both grew at a faster linear rate than did the body. The opposite appeared to be true during slow growth.

For purposes of estimating body growth from calcified structures ("back calculation"—see Smith 1983), it has frequently been assumed that growth of the structure is isometric or can be mathematically transformed so that it appears to be. However, considering the seasonal cycle of northern pike (Fig. 1C), isometric growth was only a transitional stage that rarely, and possibly never, occurs. During rapid growth, growth of both structures was positively allometric; during slow growth it was negatively allometric. This relationship was always more extreme in scales than in cleithra. These allometric growth differences substantiate that when interpreting growth from a structure, we are describing only the growth of that body part and not necessarily the growth of any other part or the fish as a whole. Growth should be compared on a relative, not an absolute, basis. Back calculation of body size at age, which has been conducted widely and not tested adequately, should be carefully reexamined and attempted only with valid ages.

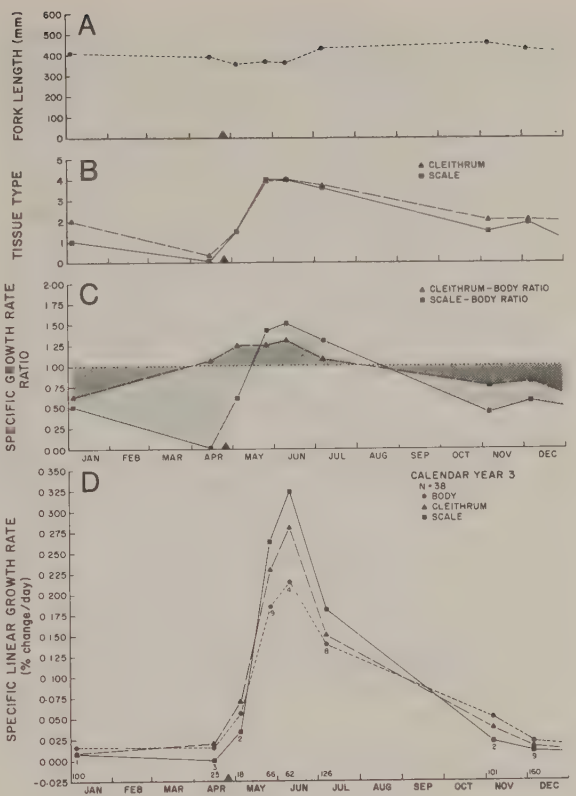


Figure 1.—Seasonal dynamics of qualitative growth of cleithral bones and scales, and quantitative linear growth of body, cleithra, and scales of 38 northern pike, *Esox lucius*, in calendar year 3 from Smoky Hollow Lake, Ontario. Sexes are combined: 26 males and 12 females. Results are averaged by month of midpoint of the mark-recapture period and plotted on the mean day, except the samples for May which are separated by growth rate (fast or slow). Dark triangles on the X axes indicate the spawning period. A) Mean fork length of pike for the mark-recapture period. B) General classification of the type of calcified tissue deposited during the mark-recapture period. As the ranking of the tissue type increases, the associated circuli on the scales appear more uniform and widely spaced, and bony tissue in the cleithra appears more opaque (Casselman 1978). A check or translucent zone of type 1 is usually associated with annuli, and type 2 with pseudoannuli. C) Relative growth of calcified tissue and body. A ratio of 1.00 indicates isometric growth (dotted line). Shading indicates the deviation from isometric growth (dark—cleithrum; light—scale). D) Specific or instantaneous linear growth rates of body, cleithra, and scales during the mark-recapture period. Number of individuals is indicated below the data set. The number of days in the mark-recapture period is indicated on the X axis.

VALIDATION

Numerous methods have been used in an attempt to validate age assessment of fish (Brothers 1979, 1983). However, most of these methods are indirect. The most powerful direct evidence for testing validity is obtained by examining structures from known age fish, e.g., stocked fish (Cable 1956), or partly known age fish that have lived in the natural environment or have been reared in captivity under natural or seminatural conditions. Partly known age fish are those that have been captured, marked (e.g., tag and fluorochrome label), and released, then subsequently recaptured so that the duration of the mark-recapture period is known. Although fluorochrome labeling is one of the most precise ways to perform this test (Casselman 1978), it is possible to remove some structures, e.g., scales and fins, at time of tagging and compare them with those obtained at time of recapture. It is also sometimes possible to see "handling marks" on structures when fish are recaptured. Although these artificial labeling techniques have been known for many

[5]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

years, they are only now becoming more widely used in fisheries. For fish from the oceanic environment, the tetracycline method of validation has been used for centra (Holden and Vince 1973; Gruber and Stout 1983) and otoliths (Beamish and Chilton 1982). Wild and Foreman (1980) also used tetracycline to validate the occurrence of daily microstructure in otoliths of tunas.

Validation of age assessment should be a routine part of every study. Because a method has been shown to be valid under certain circumstances and for certain species, it does not necessarily mean that it can be assumed to be valid under all conditions. A different set of circumstances, such as change in growth rate, would necessitate a reevaluation of the method.

Many of the methods used in validation are often used independently to assess age. Some are strongly circumstantial but still provide good corroboratory evidence. Some of these, such as length-frequency and modal progression analysis, have been used to examine the validity of assessments made on oceanic pelagics (Yabuta and Yukinawa 1957; Le Guen and Sakawaga 1973).

One procedure frequently used to check assessed age is to compare ages assessed independently from different calcified structures from the same fish. The structures most often used in age and growth studies of the large oceanic pelagics are illustrated in Figure 2, and a thorough description of these, along with collection procedures, is provided by Prince and Lee (1980).

Comparisons of these types do not validate age assessment; they simply provide a measure of agreement and give some indication of the degree of confidence that can be placed in the interpretations. This comparative procedure would be better termed verification.

Numerous studies have shown that when ages of different structures are compared, perfect agreement over a broad range of ages is unlikely. This is especially true for muskellunge, *Esox masquinongy*, when scales are involved (Fig. 3). Cleithral age assessment of muskellunge from the St. Lawrence River has been validated over the entire age range of the species (Casselman[6]). Ages attained from scale interpretations agree well with cleithral interpretations up to approximately age 10. However, in older fish, scales contain fewer recognizable annuli than do cleithra. The edges of these scales appear eroded and have characteristics indicating resorption (Casselman 1979).

Much of the evidence used to evaluate the reliability of various methods and structures has come from verification studies rather than from direct validation (Beamish and McFarlane 1983). From these verification studies, it appears that sections made from otoliths (along the line of maximum growth) are most reliable, whereas scales, especially those from older fish, are least reliable. Other structures and methods of interpretation appear to fall intermediate in these comparisons. Johnson (1983) found sections of centra and first dorsal spines of little tunny, *Euthynnus alletteratus*, to give good agreement (96%). Lee et al. (1983) indicated that vertebrae tend to underestimate age, and enumeration of all zones in otolith sections seemed to overestimate the age of Atlantic bluefin tuna. However, the interpretations of vertebrae more closely approximated the partly known age of one very old giant bluefin tuna.

Although the tendency in verification studies has been to assume that the structure that provided the oldest assessment was the most reliable, this may not always be the case. Lee et al. (1983) were unable to reject the hypothesis that two translucent zones were deposited in the otolith of Atlantic bluefin tuna each year after first maturity. Otoliths of other species have also been shown to contain multiple zonation, e.g., lake

[6]Casselman, J. M., Research Scientist, Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0. Unpubl. data, 1976.

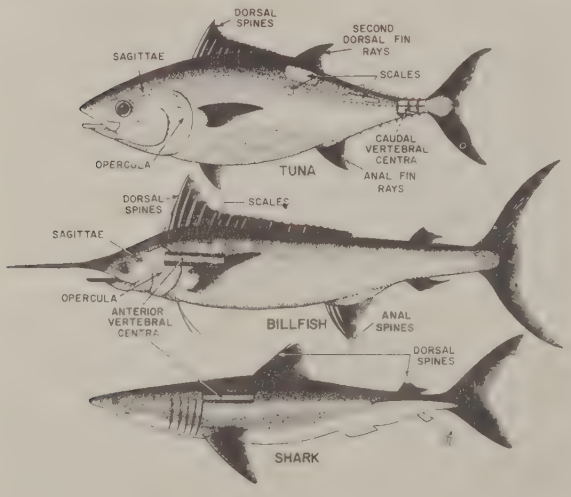


Figure 2.—Calcified structures commonly used in age estimation and verification and growth assessment of the large oceanic pelagic fishes—tunas, billfishes, and sharks. The approximate location of the sagitta in the cranium is illustrated. In addition to these hardparts, the crystalline lens of the eye, teeth, scutes, maxillaries, pterygiophores, cleithra, and various other flat bones, especially those of the opercular series, are used in some freshwater species.

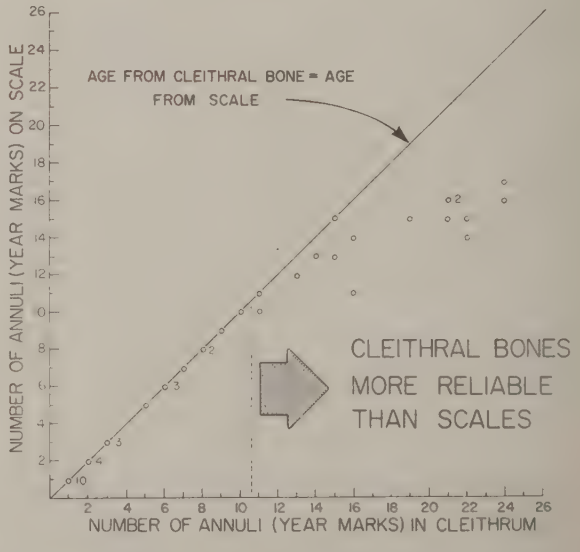

AGE FROM CLEITHRAL BONE = AGE FROM SCALE
CLEITHRAL BONES MORE RELIABLE THAN SCALES
NUMBER OF ANNULI (YEAR MARKS) ON SCALE
NUMBER OF ANNULI (YEAR MARKS) IN CLEITHRUM

Figure 3.—Relation between number of annuli on the scales and in the cleithra of 42 muskellunge, *Esox masquinongy*, collected during 1965-75 from the St. Lawrence River, Ontario. When the data set represents more than one individual, the number is indicated. For fish older than age 10, the cleithral bones are more reliable for age assessment than are the scales.

herring, *Coregonus artedii* (MacCallum[7]), and European eels, *Anguilla anguilla* (Deelder 1981), which if interpreted literally, overestimated the "true" age of the fish. In verification studies, the simple recognition of more checks or zones associated with "annuli," hence older age, may not necessarily make the method or structure better.

One of the major problems with verification studies, as currently practiced, is that the results of comparison depend entirely upon the methods used for interpreting age. Specific criteria for recognizing annuli should be provided so that bias associated with interpretation can be evaluated.

Growth data obtained from age assessments should be credible. Compilation of data sets of size at scale age published for four species of fish from the province of Ontario provided evidence suggesting that older lake trout, *Salvelinus namaycush*, had been underaged by the scale method (Casselman[8]). When these growth data were applied to the von Bertalanffy growth model, the resulting parameters for mean asymptotic fork length for northern pike (98.3 cm); walleye, *Stizostedion vitreum*, (62.2 cm); and lake whitefish, *Coregonus clupeaformis*, (56.8 cm) were realistic (Fig 4). However, the mean asymptotic fork length of 145.3 cm for lake trout was unrealistic and overestimated the maximum observed length (Martin and Olver 1980) by approximately 26%. Considering the age range of these lake trout data and the ecology of the species, the von Bertalanffy growth model should apply. This model will not apply if fish grow through several growth stanzas later in life because of a change in diet or environment, e.g., eels, *Anguilla* sp. (Sparre 1979), or if the older individuals undergo a major increase in growth rate because of increased exploitation of the population. It is possible that some of these conditions might have affected these fallacious results for lake trout. However, the principal reason is that the scale method underestimates the age of old lake trout (Casselman unpubl. data 1982). After approximately age 6, the scale method applied to lake trout fails and with increasing age, this species is increasingly underaged by this method. This alone could explain the undiminished growth of older lake trout and the resulting unrealistically high asymptotic length.

If the assessed scale ages of lake trout are corrected by verification using other calcified structures, then asymptotic length can be reduced by 25% (Casselman unpubl. data 1982). This is almost exactly the same amount by which the mean ultimate length of lake trout exceeded the observed values.

Unless it has been validated, the scale method should not be used for precise analyses of year-class strength and mortality rates of older individuals. In general, but depending upon growth rate, the scale method in freshwater fish is increasingly inconsistent from assessed ages 6 to 10. Beyond these ages, results are increasingly biased, tending towards an underestimation of "true" or calendar age. The scale method, however, is adequate for heavily exploited populations because these usually contain young, fast-growing individuals.

[7]MacCallum, W., Assessment Unit Leader, Lake Superior Fisheries Assessment Unit, Ontario Ministry of Natural Resources, 435 James Street, Box 5000, Thunder Bay, Ontario, Canada P7C 5G8, pers. commun. 1982.

[8]Casselman, J. M. 1982. Growth response to over-exploitation. Unpubl. manuscr., prepared for Report of Strategic Plan for Ontario Fisheries, Working Group No. 15. Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0.

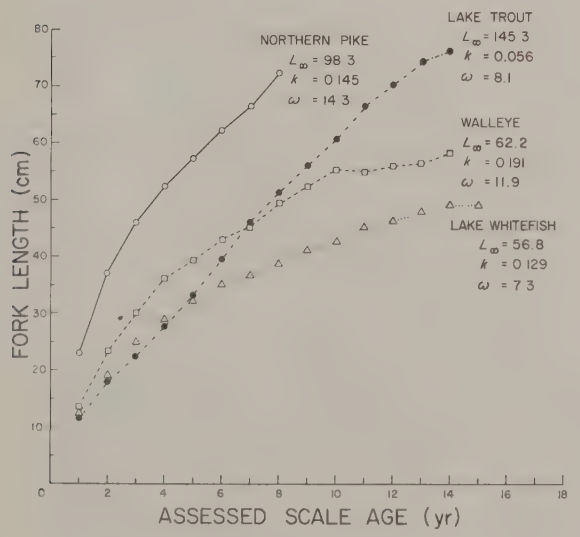


Figure 4.—Mean fork length at assessed age estimated by the scale method for four species of freshwater fish. The von Bertalanffy growth parameters are provided (k = growth coefficient, L_∞ = asymptotic length). $\omega = k \cdot L_\infty$ (Gallucci and Quinn 1979). Size-at-age data are the means of published data from numerous Ontario populations: Northern pike, *Esox lucius*, N = 18; lake trout, *Salvelinus namaycush*, N = 42; walleye, *Stizostedion vitreum*, N = 38; and lake whitefish, *Coregonus clupeaformis*, N = 15.

COLLABORATION

Often, it is not possible to validate or even use different structures to verify age assessments. In such cases, several interpretations should be made to increase the precision of the estimate. Ideally, this replication should be done by several different interpreters. Repeatability provides a measure of the degree of confidence or reliability that can be placed in the assessments; this can be expressed as the "index of concurrence" —frequency of occurrence of the modal age (Casselman et al.[9]). If examinations are conducted by several interpreters who routinely assess age of the species by similar methods, then such collaboration can provide material that is "reliably" aged. Such exchange programs have been reported for otoliths (Blacker 1974) and sections of dorsal fin spines (Antoine et al. 1983).

In the past, it was difficult to transfer the information associated with each interpretation, hence the results were usually analyzed and summarized only in terms of age. If the exchanges depend upon an examination made directly from specimens, then the program is time-consuming, and if samples such as scales are supplied, different interpreters may use different specimens. Blacker (1974) and Antoine et al. (1983) eliminated these problems by circulating photographs that could be examined and annotated so that the interpretations could be related directly to the images used.

Microfiche reader-printers, which produce photographic prints directly from structures or their thin sections, have facili-

[9]Casselman, J. M. et al. (+ 13 participants). 1980. An exchange program used to examine the scale method of ageing yellow perch (*Perca flavescens*) from Lake Erie. Unpubl. manuscr. of an International Exchange Program, 136 p. Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0.

tated such exchange programs. Prints from these machines can be made quickly and easily, and are relatively inexpensive. The Recordak Magnaprint Reader (Model PE-1A by Eastman Kodak, Rochester, N.Y.) uses the wet silver method to produce a negative image (Fig. 5) that is as good as those obtained by normal photographic procedures. These prints are of high resolution; even photocopies of the images are clear and have good contrast, and can also be interpreted easily. This hard copying technique can be used to obtain a permanent record of the interpretation to test consistency within and between interpreters and within and between samples and studies. Annotated hard copies, which document the interpretation and assessment, should be prepared routinely. These permanent records would make it possible to make corrections without reinterpreting the samples, if subsequent validation or verification proved the interpretations were incorrect or biased. These prints can also be used as training aids.

This hard copying procedure makes collaboration easier and more convenient. By circulating photocopies, collaborators can independently interpret, mark, and annotate the images. People seem more willing to participate in exchange programs and respond quickly when hard copies are used. Results can be more easily summarized and circulated, so that inconsistencies can be detected quickly.

Illustrated in Figure 6 are the summaries of the interpretations obtained in an international exchange program for a scale from a yellow perch, *Perca flavescens*, from Lake Erie (Casselman et al. footnote 9). In this particular exchange program, opercula and otoliths were used to verify the assessed scale age after the interpretations had been completed (Fig. 7). Although there was good agreement in the assessed age among the interpreters in this exchange program, there was some disagreement on the precise location of the annuli (e.g., Fig. 6, interpreter M, second annulus). Hence, different growth patterns were assigned to the same fish. Such exchanges demonstrate the types of problems that occur in routine age assessment. For example, in this study there was also considerable disagreement over the interpretation of the edge of the scale, a common problem when interpreting age from calcified structures.

Workers routinely conducting age assessment on the same species should participate in cooperative exchanges to standardize and test procedures. Now that quick and easy hard copying methods are available, the details of specific interpretations can be transferred easily and precisely.

Figure 5.—Photographic print of the scale impression of a lake whitefish from Lake Mindemoya, Ontario. Fish was 361 mm total length, 320 mm fork length, and 460 g total weight. Estimated age 6+. Print is a negative image (10X) made directly from the scale used as a negative.

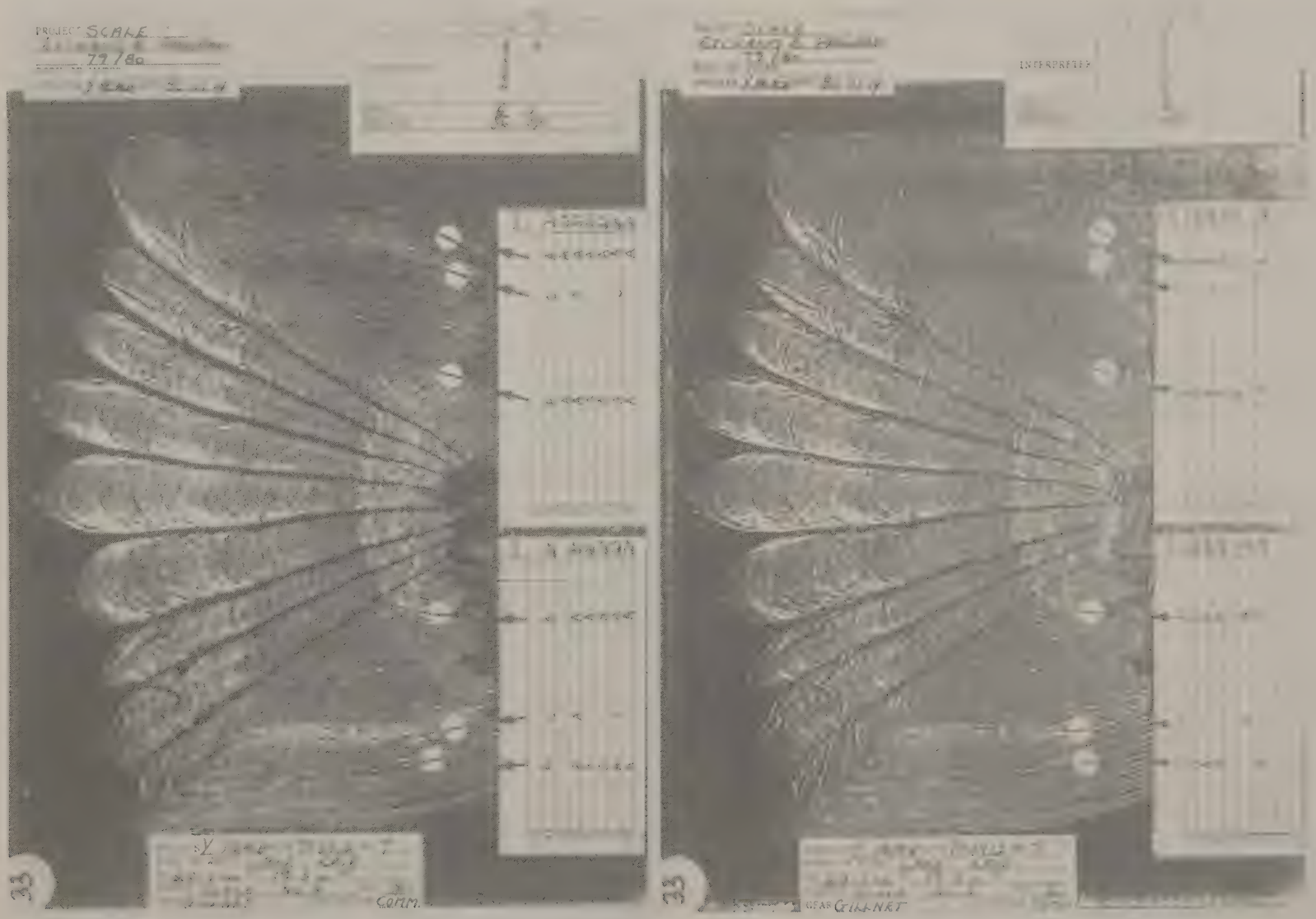
AUTOMATION

Age and growth determination of fish will not become a truly objective science until the interpretation is quantified (see section on Interpretation) and the process can be mechanized and automated. Systems have already been developed to mechanize enumeration of circuli on scales (Mason 1974) and to automatically recognize checks and zones in scales and other structures by image analysis (Fawell 1974). Mechanization and automation of the scale method will not be accomplished easily because scales contain many types of checks that have been associated with annuli, and these vary in appearance throughout the different regions of the scale. However, other calcified structures, which contain fewer types of translucent zones associated with annuli, lend themselves more easily to automated procedures.

Although the most logical approach would be to use optical density in an automated system, a thorough understanding of the physical and chemical differences among checks on scales and among optically different zones in other structures could provide insight into differences that might be applied to detect seasonal growth patterns and perform automated analyses. The electron microprobe X-ray analyzer has substantiated that the translucent zone in fish calcified tissue is more heavily mineralized than adjacent opaque zones (Casselman 1974, 1978), and that calcium content is directly related to translucency (Fig. 8). Hence, in addition to optical zonation, fish calcified tissue contains corresponding chemical zonation. Even when calcified tissue appears to be optically uniform, elemental zonation corresponding to age can be shown to exist (Fig. 9). Microprobe analysis has also been used in analyzing centra of the spiny dogfish, revealing that calcium zonation occurs even in the relatively cartilaginous skeletons of sharks (Jones and Geen 1977). Although this method suggests possibilities for automation, line scan analysis with the electron microprobe is time-consuming and expensive.

Since fish calcified tissue shows mineral zonation and density that are directly related to translucency, X-radiography could be applied. Centra of elasmobranchs show differential zonation when X-radiographs are prepared by soft X-ray techniques (Cailliet, Martin, Kusher, Wolf, and Welden 1983).

Figure 6.—A summary of the interpretations of the scale of a yellow perch, *Perca flavescens*, resulting from an international exchange program (Casselman et al. text footnote 9) used to evaluate scale age assessment of perch from Lake Erie. Assessments were originally made on negative images printed at 38X magnification. Results for interpreters B to H on top scale image and I to N on bottom scale image. The positions of all annuli (A) and checks (C) marked by each interpreter are indicated. Specific data on the fish and the program are superimposed on the prints (24X).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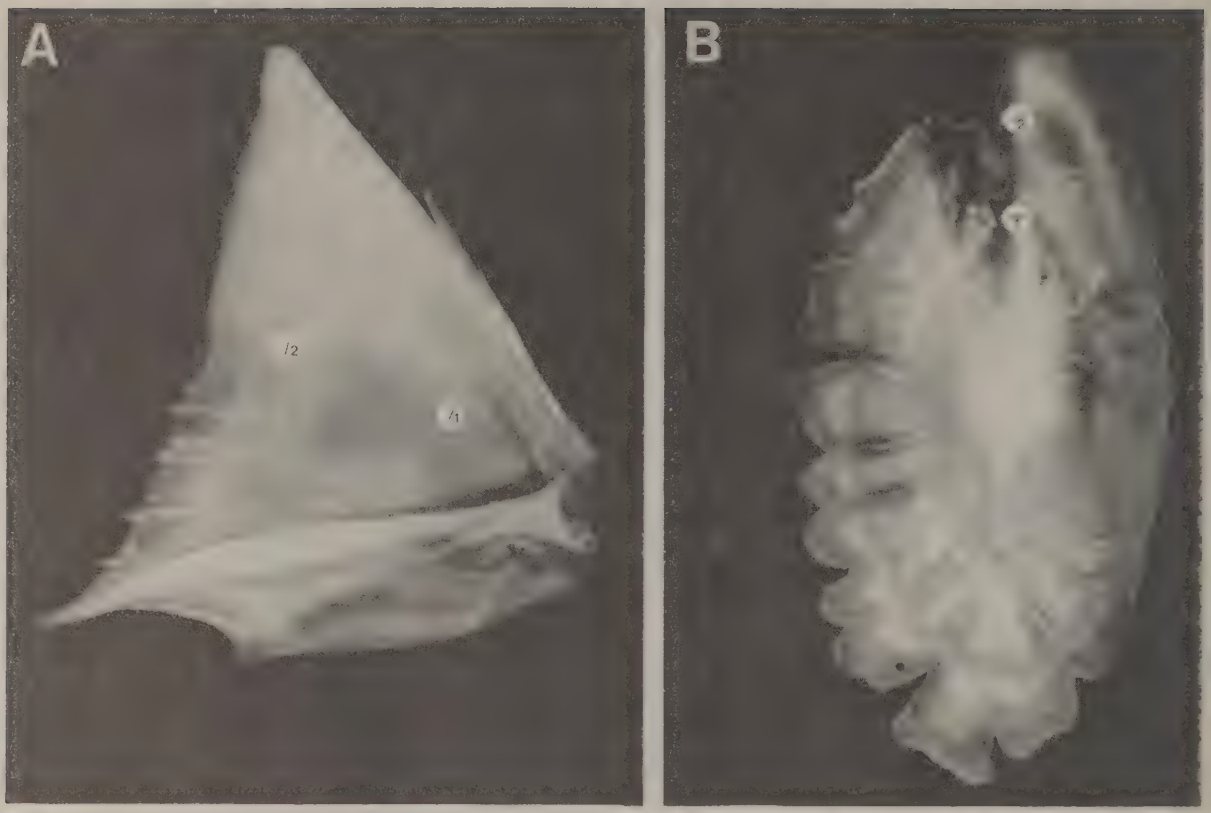

Figure 7.—Other calcified structures used to corroborate age assessment of the scales used in the exchange program for Lake Erie yellow perch. A) Opercular bone (3X); B) Whole otolith (11X). Both structures are from the same fish for which scales are illustrated in Figure 6. Reflected light.

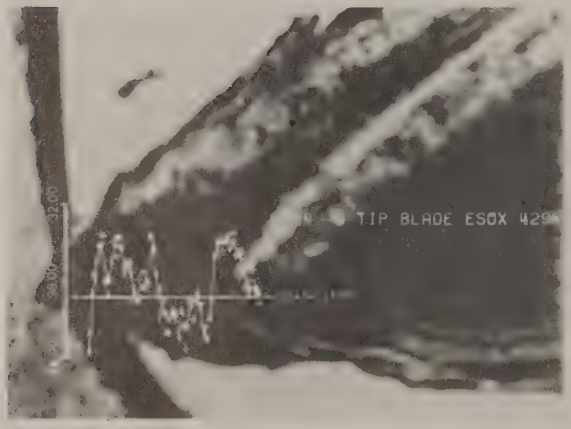

Figure 8.—Electron microprobe X-ray analysis for calcium across the optically different zones of a calcified structure. Calcium concentration (percent dry weight) determined by line scan analysis across the sixth translucent zone, seventh opaque zone, and seventh translucent zone (on the edge) of a thin transectional slice (thickness 158 μm) of the tip of a cleithrum of a northern pike. Actual distance of scan line is 900 μm. Transmitted light.

X-ray densimetric techniques have been used in dendrochronology, and systems have been developed that have totally automated and computerized tree-ring analysis and the age assessment of trees (Parker et al. 1973). Densimetric scanning techniques are directly applicable to age and growth assessment of fish because the optically different zones in fish otoliths and bony structures are also X-ray densitometrically different, and are remarkably similar to early and late wood in tree-ring formation (Fig. 10). This highly developed technology has been applied successfully to osseochronology of fish (Casselman et al.[10]). A typical example of an X-ray density scan of a radiograph made in a Hewlett Packard Faxitron Series X-ray System from a fin ray section of a lake sturgeon, *Acipenser fulvescens*, is shown in Figure 11.

[10]Casselman, J. M., M. L. Parker, and L. A. Jozsa. 1981. Osseochronology using X-ray densitometric techniques. Unpubl. manuscr. Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0.

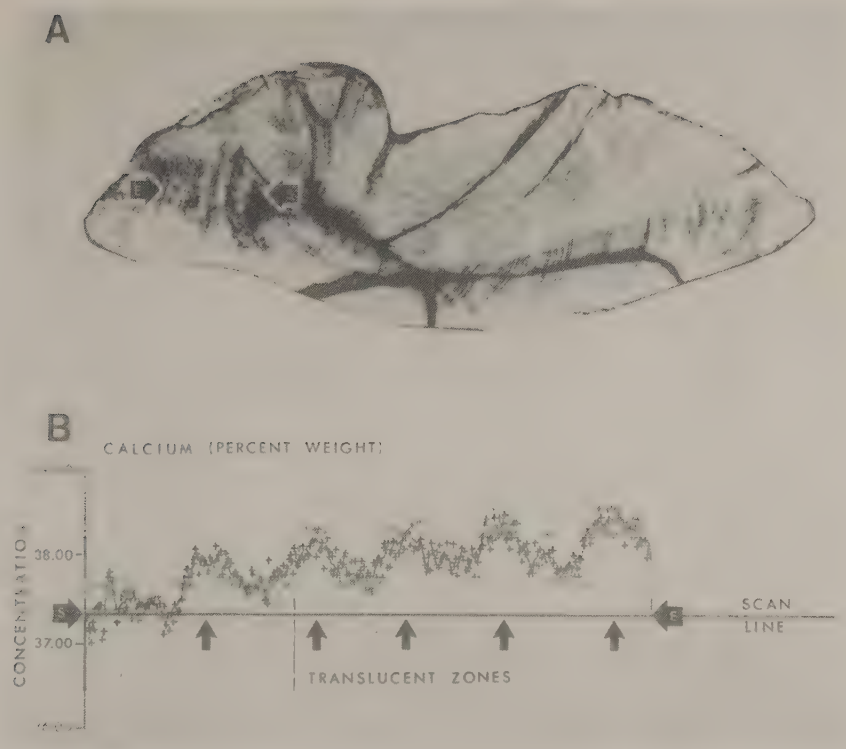

Figure 9.—Electron microprobe X-ray analysis for calcium across a relatively translucent area of an otolith (sagitta) of a European eel, *Anguilla anguilla*. A) Cross section from the middle of the otolith of a fish from the Shannon River, County Tipperary, Ireland. Fish was 712 mm TL, 746 g TW. Arrows indicate the start and end of line scan analysis. Transmitted light; thickness 200 μm (59X). B) Calcium concentration as determined by line scan analysis. Arrows indicate the starting point (S) and end (E) of the scan, and correspond to those illustrated in A.

INNOVATION

In recent years, several innovative techniques have been applied to the problem of age determination. Otoliths of many species have been shown to have microstructure that suggests daily rhythmicity (Pannella 1974, 1980; Brothers 1979). Validation of the occurrence of these daily growth increments (Brothers et al. 1976; Taubert and Coble 1977; Wild and Foreman 1980; Radtke and Dean 1982) now makes it possible to examine and more precisely verify interpretations made by other, more subjective means.

Recently, daily increments in the otolith microstructure have been enumerated to verify the yearly periodicity of an "annulus" in the otoliths of largemouth bass, *Micropterus salmoides* (Taubert and Tranquilli 1982), and fallfish, *Semotilus corporalis* (Victor and Brothers 1982). Several techniques such as video enhanced light microscopy (Brothers et al. 1983) and scanning electron microscopy (Radtke 1983) have been used to increase the resolution of this microzonation. Acetate replication of the ground and hydrochloric acid etched surface of the otoliths provides useful imagery (Wild and Foreman 1980). Although the resolution is not as good as that obtained by electron microscopy, it is adequate and less costly. Detailed studies of otolith microstructure using electron microscopy and radioisotopes (e.g., ^{45}Ca) will help elucidate the physical properties of microzonation (Watabe et al. 1982) and the physiological factors controlling incremental growth (Mugiya et al. 1981; Tanaka et al. 1981).

Acetate replication has been used on otoliths of American eels, *Anguilla rostrata*, to provide new insights into the problem of age assessment of this species. It has been extremely difficult to interpret age of eels from the upper St. Lawrence River and Lake Ontario by using standard otolith procedures. By combining acetate replication and electron microprobe analysis of eel otoliths, it is now possible not only to assess the age more easily and consistently but also to describe the chronology of eel migration from the sea (Casselman 1982). Three types of optically different zonal patterns are observed in the acetate replicas (Fig. 12). One type is associated with the NUCLEUS and is comprised of broad, opaque zones separated by two to four translucent zones. Strontium-calcium ratios as determined by electron microprobe analysis substantiated that this tissue is deposited in the marine environment. Outside the NUCLEUS is a set of zones referred to as the zones of TRANSITION that are associated with migration up the St. Lawrence River. The opaque zones in this region are narrow and are separated by two or three broad translucent zones. Outside this region is the EDGE, which contains numerous broad opaque zones separated by very distinct, narrow translucent zones. The first opaque zone outside the zones of

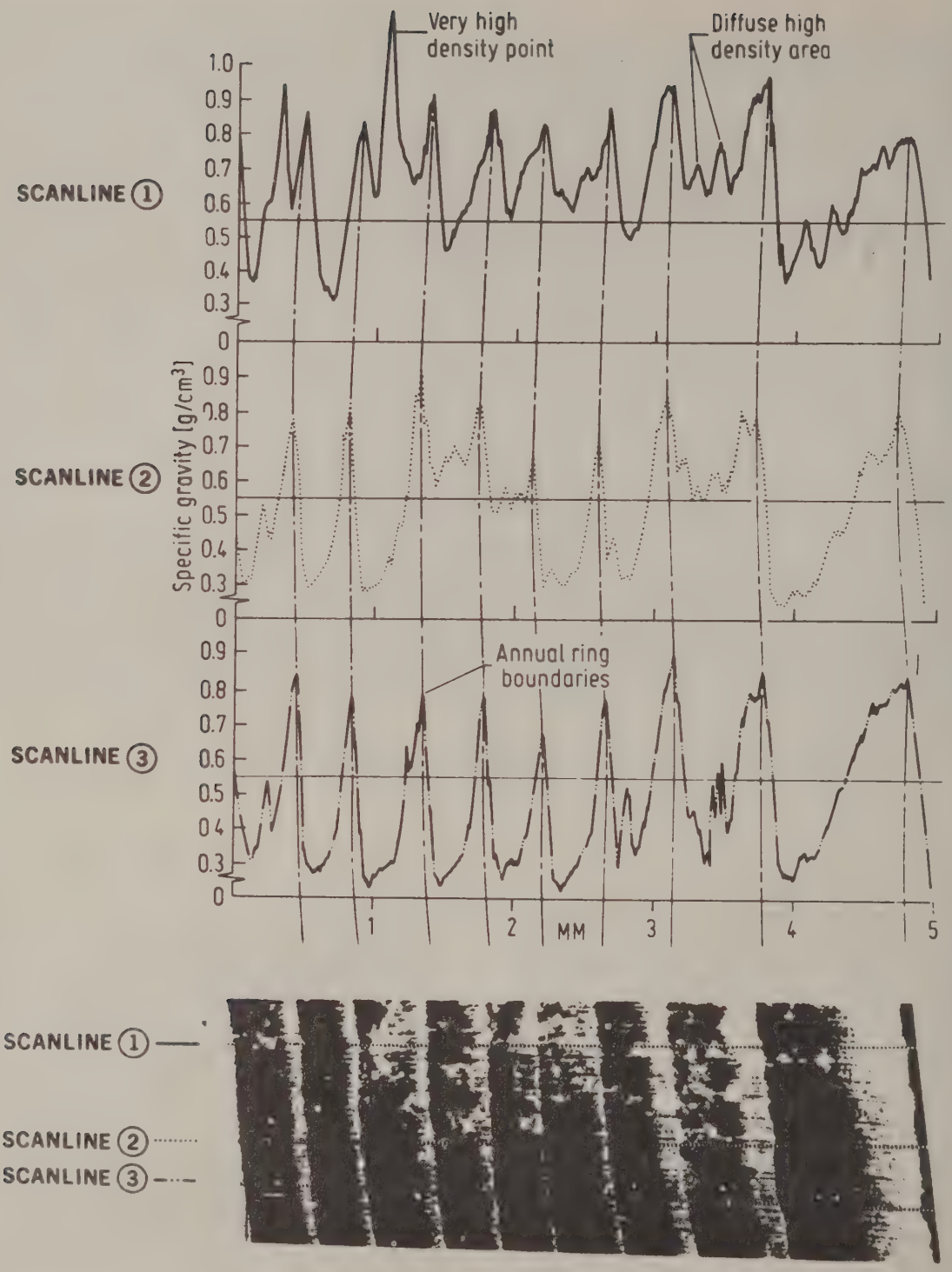

Figure 10.—Tree-ring density plots of three radial scans across a radiograph of a 2 mm thick transverse section of western hemlock, *Tsuga heterophylla*. The plots show the intra-ring density patterns of nine annual rings, as well as the relative density of two types of wood (earlywood—low density, and latewood—high density). Reproduced from Parker et al. (1974), figure 3, from Wood Science and Technology by permission of the authors and Springer-Verlag.

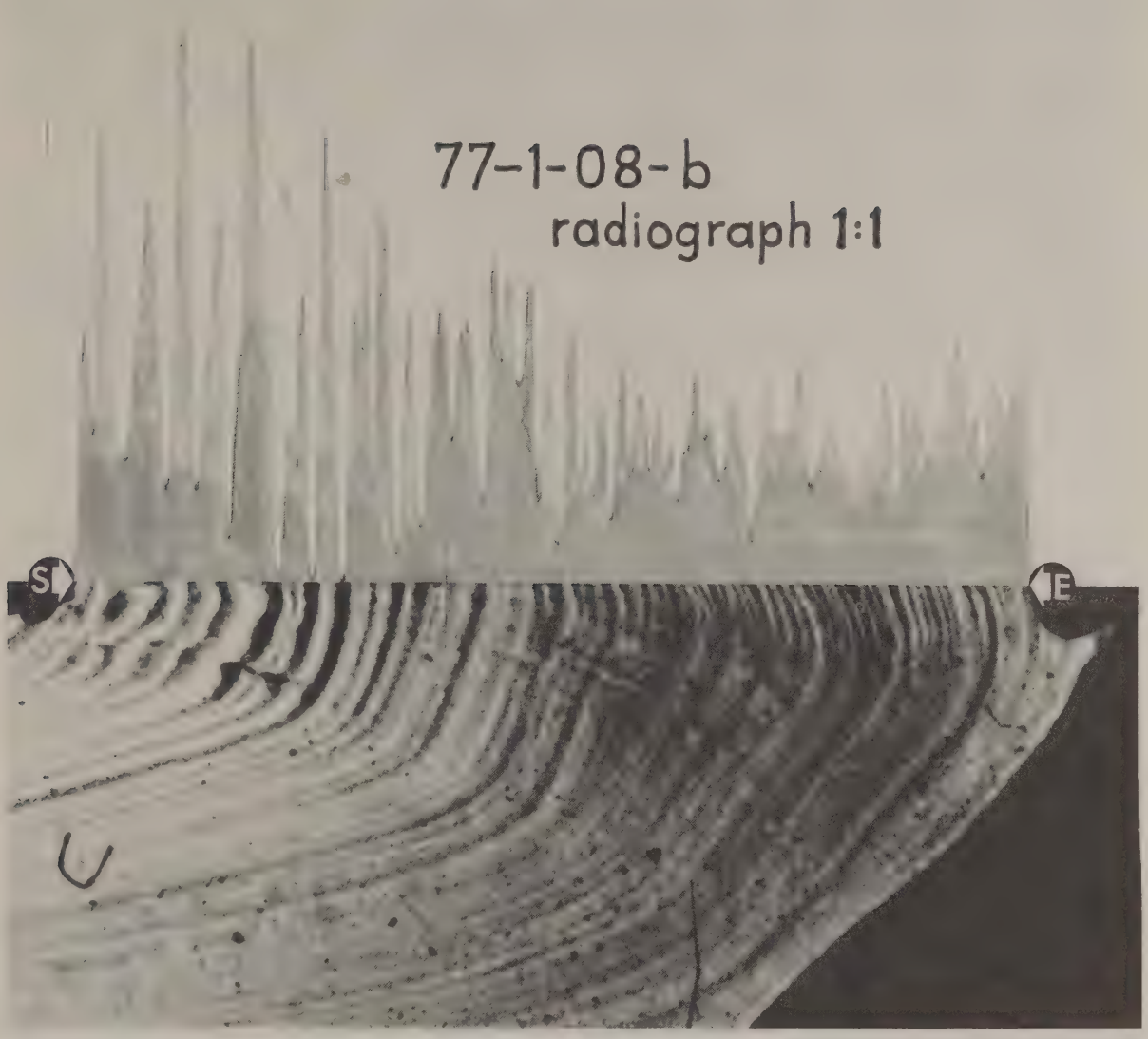

Figure 11.—Calcified tissue density plot of a radial scan across a radiograph made from a portion of a thin cross section (250 μm) of the first pectoral fin ray of a lake sturgeon, *Acipenser fulvescens*, from the St. Lawrence River. Fish was 160 cm TL, 44 kg TW, age estimated at approximately 60 yr. Zonation appears as it would in the fin section if it were viewed in transmitted light (25X). Light colored zones on the radiograph correspond to the translucent zones and have a high X-ray absorption and high density. The arrows mark the starting point (S) near the nucleus and the end (E) of X-ray density scan near the edge of the fin.

TRANSITION is usually narrower than are subsequent opaque zones. This region is associated with life after migration either in the upper St. Lawrence River or Lake Ontario. Strontium-calcium ratios in this region of the otolith indicate life in the freshwater environment. The translucent zones associated with the EDGE are very distinct and easily recognized, so, by using acetate replication, it is now possible not only to assess the age of eels more reliably but also to determine precisely how long the fish has spent in each particular habitat. Strontium analyses helped Bagenal et al. (1973) discern similar migration information for brown trout, *Salmo trutta*. Calcified tissue contains valuable chemical information that can be used to improve and refine the interpretation of environmental growth history of fish.

Innovative biochemical methods have been developed to measure "instantaneous" growth rate of calcified tissue. This technique, which can be referred to as "scale growth index," measures the uptake of ^{14}C-glycine incorporation by cells associated with isolated scales (Ottoway and Simkiss 1977, 1979; Adelman 1980). Although the technique and equipment are sophisticated, the method has potential for studying the factors causing check formation through a study of the growth rate of the scales.

Some biochemical techniques appear potentially useful for assessing age. One method, which is based on precise changes in the amount of insoluble protein in the crystalline lens of the eye, consists of two procedures: 1) Obtaining the appropriate lens fraction, and 2) quantitatively analyzing its protein com-

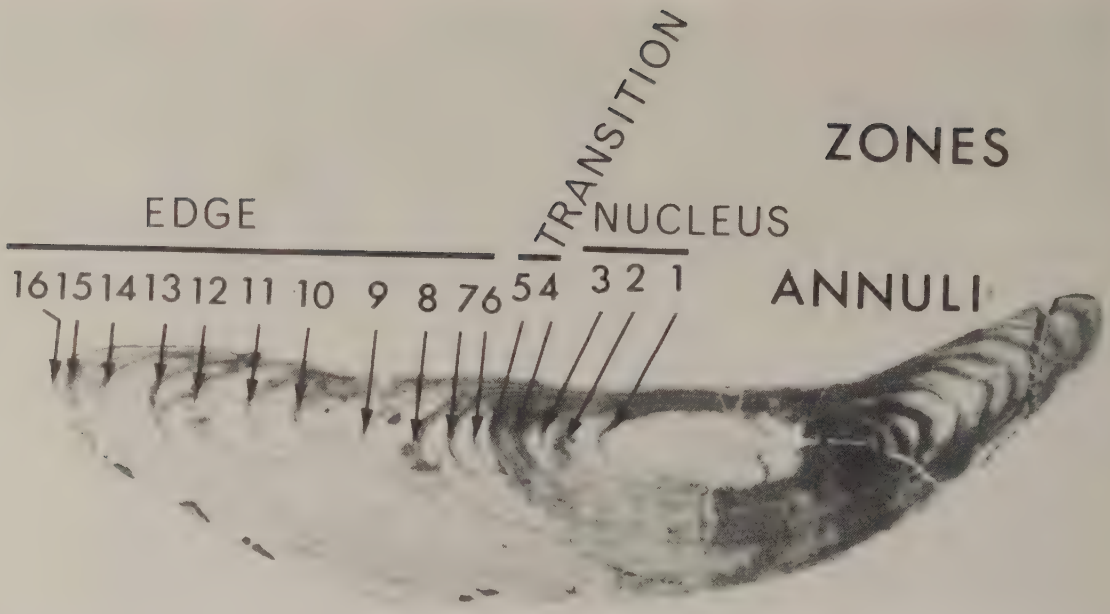

Figure 12.—Photomicrograph of a cellulose acetate replica of a longitudinally ground and acid etched otolith (sagitta) of an American eel, *Anguilla rostrata*, from the upper St. Lawrence River. Fish was 800 mm TL, 1,210 g TW, assessed age 16 yr. Zonation appears as it would on the otolith surface if it were viewed in reflected light (50X). The zones (translucent) associated with annuli are indicated and are separated into the three distinct types of zonation. The first three translucent zones are of a similar type and are associated with the NUCLEUS. The fourth and fifth translucent zones are similar, broad, and different from preceding and succeeding zones, and are associated with TRANSITION, or migration up the St. Lawrence River (Casselman 1982). The remaining 10 translucent zones are similar, narrow, and are associated with the EDGE.

position (Otero and Dapson 1972). Another involves amino acid racemization (Helfman and Bada 1976; Helfman et al. 1977; Masters et al. 1977), which consists of a comparison of the L and D isomers of aspartic acid. Proteins are initially comprised almost exclusively of L-amino acids. However, these change with time into their D-enantiomers at a rate that is proportional to temperature. With this technique, it would be necessary to know the thermal history of the fish. This may be possible in the future.

Radioactive geochronology, which utilizes natural radionuclide ratios, appears to be one of the most potentially useful new techniques. This method has been used to examine growth rate of marine clams (Turekian et al. 1979; Turekian and Cochran 1981). Radiometric age determination has been used recently to confirm the longevity of splitnose rockfish, *Sebastes diploproa*, by measuring uranium decay series nuclides ^{226}Ra and ^{210}Pb in otoliths (Bennett et al. 1982). This is a truly objective procedure and signals that innovative techniques may revolutionize age and growth assessment of fish in the future.

The calcified structures of fish contain a great deal of additional information that should not be overlooked. They have been valuable tools in stock identification (Ihssen et al. 1981). Objective methods now exist for stock separation using Fourier series analysis to quantify the shape of calcified structures (Jarvis et al. 1978; Casselman et al. 1981). Characteristics in the calcified structures are strongly influenced by environmental conditions, but a genetic basis exists. Some calcified structures, such as scales, are probably more strongly influenced by environmental conditions than are others, such as otoliths (Casselman 1978). This may explain why otolith shape is a better discriminator than scale shape for lake whitefish stocks (Casselman et al. 1981).

CONCLUSIONS

From this overview of the procedures, problems, and progress in assessing the age and growth of fish from their calcified structures, it is apparent that this science is now expanding rapidly and is going through a technological revolution. The techniques and tools, especially those pertaining to interpretation, validation, and automation, are becoming much more powerful and sophisticated. The problems have changed little, but the practical application is rapidly being improved, refined, and expanded. The wider use of fluorochrome markers, e.g., tetracycline, will greatly improve interpretation and will provide badly needed tests of validity for not only age assessment, but also growth evaluation. The major advances in microelectronics and computer technology in recent years signal that automated interpretation is feasible and inevitable. Although the procedures of age and growth assessment of fish from their calcified structures have remained virtually unchanged over the past 50 yr, there are now signs that the technology is starting to undergo major changes and is becoming increasingly specialized. There is evidence that the techniques and tools used in the future may be radically different from those used today. Innovations are being developed, such as biochemical methods of measuring instantaneous scale growth to provide a direct measure of growth rate, and radiometric age determination to provide a more objective age assessment. Such procedures could eventually eliminate subjectivity and

make age and growth determination a truly objective science. Calcified structures also contain valuable information that can be applied to other fisheries problems. The quantification of the shape of calcified structures provides a powerful tool that permits stock identification from materials that are routinely collected for age and growth purposes.

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Some Statistical Characteristics of Ageing Data and Their Ramifications in Population Analysis of Oceanic Pelagic Fishes

JOSEPH E. POWERS[1]

ABSTRACT

The statistical characteristics of age estimates in relation to their use in population dynamics are examined by means of Monte Carlo simulation. Error structures in mortality and length-age models are discussed in relation to the trade-offs between increasing the precision in ageing an individual fish versus increasing sample size. Some heuristic rules for making this decision are given, in addition to examples using vital rate parameters common in oceanic pelagics. Biases in age estimation lead to varying degrees of bias in vital rate estimates. However, for these simulations, if the error in the age estimate is < 10%, then it appeared that errors in rate estimates were best reduced by increasing sample size rather than increasing precision of ageing techniques. The choice of an ageing technique should be made in the context of the statistical properties of the vital rates.

INTRODUCTION

A necessary ingredient for most population assessment models is a quantitative description of the population's vital rates, i.e., the change in the population parameter with respect to time. In many cases, these rates are measured over the life span of the fish. Therefore, the measurement of age of the fish provides the key variable of time which is needed for rate estimations, such as mortality and growth. Mortality and growth rate models provide quantitative information on the status of fish stocks and at the same time may be used in more sophisticated models, such as yield-per-recruit analysis and cohort analysis. The need for determining the age of fish as inputs to population dynamics models is well-known and, thus, a discipline of age determination of fishes has arisen.

Unfortunately, both random and systematic errors in age determination occur with existing ageing techniques (Lee et al. 1983). Additionally, biases in rate estimation may be introduced by the particular statistical procedure used (Ricker 1969). This is especially true of methods to fit the von Bertalanffy growth model to age-length data. Several procedures have been devised to reduce or alleviate these biases (Bayley 1977; Gallucci and Quinn 1979; Cohen and Fishman 1980; Bartoo and Parker 1983); but measurement errors of age may exacerbate the problem. Additionally, the normal presentation of ageing results often does not allow meaningful statistical comparisons to be made (Dapson 1980).

Random and systematic errors in age determination will probably continue to occur for some time, so we must be aware of the potential biases that they introduce into population assessment models. In this study the affect of variation and bias in age determination on growth and mortality models was examined by Monte Carlo simulations. The simulations were designed to mimic the biological parameters that are typically exhibited by oceanic pelagic tunas, billfishes, and sharks. From the simulation results, conclusions about the likely error structures are made and the ramifications for population dynamics studies of these species are discussed.

SIMULATION DESIGN

Two Monte Carlo simulators were constructed to analyze the error structure of the instantaneous rate of total mortality (Z) and the von Bertalanffy growth parameters (k and L_∞) as a function of the age (t). Using the simulations, the precision and bias of the estimators were examined. The following terms are defined for this study: Percent bias—the percentage difference between the estimated parameter and the expected value of that population parameter (expressed relative to the expected value of the population parameter), and absolute bias —the difference between the estimated and expected value. Reference to accuracy denotes the degree to which the expected and estimated values coincide, whereas, precision refers to the amount of random error that one expects in the estimate.

I assumed a stable population with continuous recruitment whose probability distribution function at age $f(t)$ was:

$$f(t) = Z \exp(-Zt). \quad (1)$$

A random sample of size N was chosen from this distribution, i.e., N animals were "aged" (t) using the simulation. I also assumed that the error in ageing was normally distributed with:

$$\text{mean} = t \cdot (100\text{-BIAS})/100$$
$$\text{variance} = (t \cdot SD)^2$$

where BIAS = the percent bias in ageing, and
SD = variation in ageing, i.e., coefficient of variation of age estimate.

Published growth equations (Lee et al. 1983) show both variance and absolute bias in age estimates to increase with age. Thus, I used the above formulation.

[1]Southeast Fisheries Center Miami Laboratory, National Marine Fisheries Service, NOAA, 75 Virginia Beach Drive, Miami, FL 33149-1099.

An individual animal's growth was depicted with the von Bertalanffy growth model:

$$L_t = L_\infty(1\text{-}\exp[-k(t\text{-}t_0)]) \quad (2)$$

where L_t = the length at age t
L_∞ = the asymptotic length
k = growth rate parameter, and
t_0 = theoretical age at which length is equal to zero.

For purposes of this simulation, we assumed $t_0 = 0$, thus

$$L_t = L_\infty[1\text{-}\exp(-kt)].$$

Using a random estimated age chosen as above, a random length was generated by Equation (2). The "measured length" $\hat{L}_t$ was generated as a normal random deviation with:

$$\text{mean} = L_t$$
$$\text{variance} = (SL \cdot L_t)^2$$

where SL = the variation in animals of a given age in the population, i.e., the coefficient of variation in the length.

An increasing variation in length at age has been shown to be common in fishes (Lee et al. 1983).

An estimate of the mortality rate (Z) was calculated using an average age method (Ssentongo and Larkin 1973):

$$\hat{Z} = N/[(\bar{t} - t_C)(N + 1)] \quad (3)$$

where $\bar{t}$ = the average age of fish in the sample, and
t_C = the age of recruitment, or, alternatively, the age of first capture.

If $\hat{t}$ (one aged individual) was $\leq t_C$, then that data point was rejected and not used to calculate $\bar{t}$ or N.

Estimates of $\hat{k}$ and $\hat{L}_\infty$ were obtained by least squares regression of $\hat{L}$ versus $\hat{t}$. If $\hat{L}$ or $\hat{t}$ was < 0, then the data pair were rejected. A simple "brute-force" regression fitting procedure was used:

$$y = ax \quad (4)$$

where $y = L_t$
$a = \hat{L}_\infty$, and
$x = 1\text{-}\exp(-\hat{k}\hat{t})$

and an iterative search was made over $\hat{k}$. The iterative search simply tested all values of k at 0.01 increments over a reasonable range of k. It was assumed that this procedure did not introduce any appreciable bias in the estimates.

The underlying population parameters Z and k were chosen to mimic rates that were similar to estimated values for a particular oceanic pelagic tuna, the northern Atlantic bluefin tuna (Parrack and Phares 1979). Since L_∞ is only dependent on the length scale, its value is not important in the simulations. Therefore, the value used for the simulation was scaled to unity.

The sample size (N) for the mortality estimation was chosen to be 10 for the base or standard case. Since samples were drawn randomly from a stable age distribution (which is seldom the case in reality), the variability produced by this small sample would be smaller than what one would encounter in a field situation with the same sample size.

Additionally, I assumed in this simulation that fish are aged precisely, i.e., that the age of a fish is measured in a fraction of a year. A few cases were simulated by assuming ageing was done in discrete annual intervals. The results indicated that this introduced more variation into rate estimates. However, computational limits required that I focus on the assumption of fractional age measurements.

The simulations were run for 200 iterations each. More iterations were used in a few test cases. The results showed that the means ($\hat{Z}$, $\hat{k}$, $\hat{L}_\infty$) and variances generated by 200 iterations of the simulation had not completely stabilized, but that the qualitative conclusions were not affected. Additionally, computational costs warranted that the iteration number be limited.

The parameter values for the base simulations are given in Table 1. Various simulation tests were performed using alternative values of Z, t_C, k, SD, BIAS, and N. The resulting relationships follow.

Table 1.—Base case input parameters for the Monte Carlo simulations of mortality and growth estimations.

Definition	Symbol	Value
Underlying total instantaneous mortality rate	Z	0.3
Age of first capture	t_C	3.0
Underlying von Bertalanffy growth rate	k	0.1
Underlying von Bertalanffy maximum size	L	1.0
Coefficient of variation of age estimates ($SE_{\hat{t}}/\hat{t}$)	SD	0.1
Coefficient of variation of length estimates ($SE_{\hat{t}}/L_{\hat{t}}$)	SL	0.2
Percent bias error in ageing	BIAS	0.0
Sample size (mortality estimation)	N	10
Sample size (growth estimation)	N	50
Accuracy of growth rate parameter	EPS	0.01
Number of simulated iterations	IMAX	200

SIMULATION RESULTS

Mortality

The simulation results showed that $\hat{Z}$ was skewed to the right, i.e., the modal outcome occurred at values of Z lower than the mean. The degree of skewness depended on the parameter conditions being simulated. The standard error of the estimate of Z increased as the coefficient of variation of the age determination method (SD) increased. The standard error was lower for lower mortality rates (Fig. 1). These results were based upon a sample size of $N = 10$; however, note that reduction in SD only marginally reduced the standard error. Substantial reductions in the standard error did not occur until the age determination variation was $< 1\%$ to 5% of the age estimate.

When the sample size increased, the standard error also declined, as expected (Fig. 2). The relative reduction in the standard error of $\hat{Z}$ with an increase in ageing accuracy was much less pronounced as the sample size became larger. Additionally, there was some bias introduced into the estimation (Table 2). Apparently, this bias was caused by the correction for small sample sizes $[N/(N + 1)]$ in Equation (3), producing an unbiased estimator of Z only when t was measured without error. As N becomes large, this form of bias decreases. Note, however, that random errors in age determination are confounded

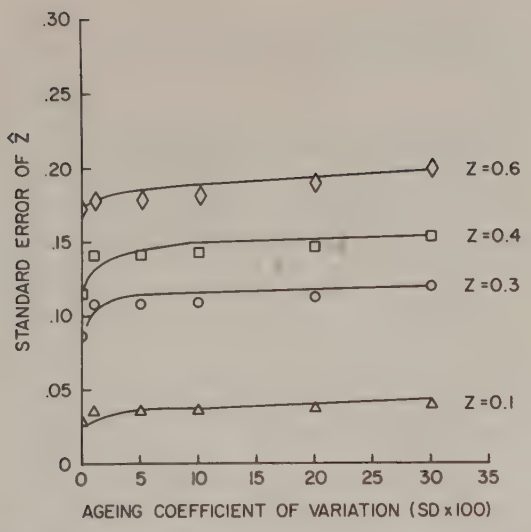

Figure 1.—Simulated effect of the percent random variation in age estimates (SD) on the standard error of total mortality rate $\hat{Z}$ for alternative values of Z (Z = 0.1, 0.3, 0.4, 0.6). Each simulated mortality estimate was based on a random age sample of N = 10.

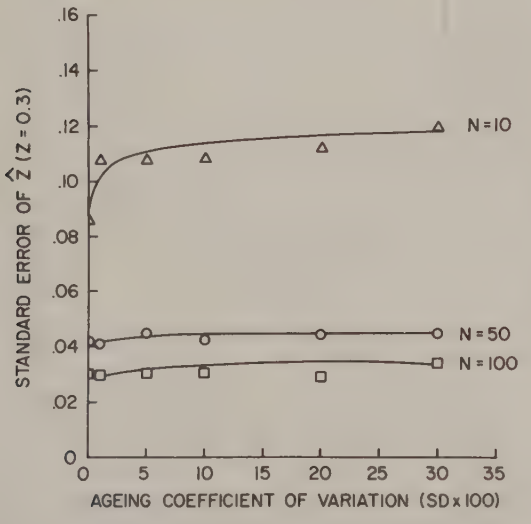

Figure 2.—Simulated effect of the percent random variation in age estimates (SD) on the standard error of total mortality rate $\hat{Z}$ for alternative sample sizes (N = 10, 50, 100). The underlying mortality rate was $\bar{Z}$ = 0.3.

Table 2.—Percent bias in estimate of $\bar{\hat{Z}}$ derived from 200 simulation runs for alternative random variations in age determination (*SD*). Sample size for estimating $\hat{Z}$ was *N* = 10.

Age variation (*SD*)	Percent bias in $\bar{\hat{Z}}$			
	$Z = 0.1$	$Z = 0.3$	$Z = 0.4$	$Z = 0.6$
0.01	3	3	1	−2
0.05	3	3	1	−2
0.10	4	4	1	−1
0.20	5	5	2	−1
0.30	6	6	2	1

with the effect of the sample size correction. Large random errors tended to cause $\hat{Z}$ to increase (Table 2).

If age determinations were biased then there were resulting biases in the estimate of the mortality rate (Fig. 3). It is interesting to note that interaction of the bias in age determination with the bias introduced by larger random errors in ageing may actually improve the estimate of mortality (Fig. 3). However, the exact relationship between these factors would be difficult to predict a priori, so there is not likely to be any practical utility of the phenomenon.

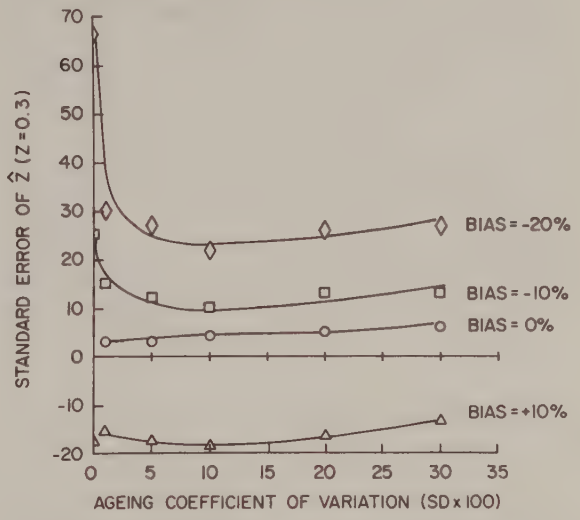

Figure 3.—Simulated effect of the percent random variation in age estimates (SD) on the percent error in the estimate of $\hat{Z}$ in relationship to Z = 0.3. The effect is shown for alternative percentages of bias (BIAS = −20%, −10%, 0%, 10%) in the age determination method. Sample size for each $\hat{Z}$ was N = 10.

Both random and systematic errors produced an error in $\hat{Z}$ (Fig. 4). To measure this error, I used the square root of the mean squared error (MSE), i.e.:

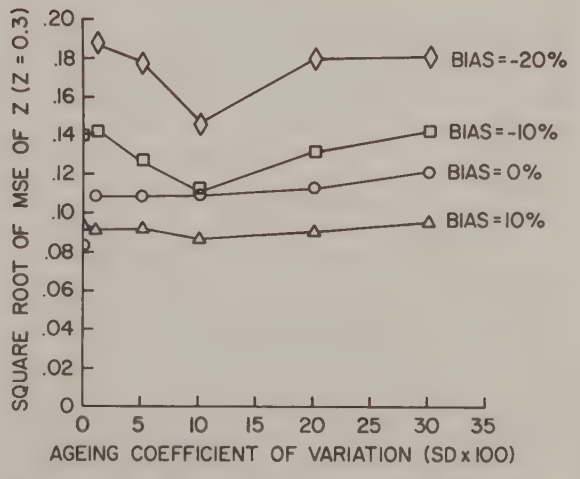

Figure 4.—Simulated effect of the percent random variation in age estimates (SD) on the square root of the mean squared error (MSE) of $\hat{Z}$ in relation to Z = 0.3. The effect is shown for alternative percentages of bias (BIAS = −20%, −10%, 0%, 10%) in the age determination method. Sample size for each $\hat{Z}$ was N = 10.

$$\sqrt{MSE} = \sqrt{[VAR(\hat{Z}) + (\hat{Z} - Z)^2)]}.$$

The results show the interplay between bias and variation, i.e., between validity and reliability of the estimate.

Growth

The growth parameters $\hat{k}$ and $\hat{L}_\infty$ tended to show distributions that were less skewed than that of the mortality rate. In most cases the simulated frequency distributions could not be distinguished from a symmetrical distribution.

Sample size and accuracy of age distribution produced the expected results in the standard error of $\hat{k}$ (Fig. 5). Increasing sample sizes and ageing accuracy both decreased the variation in $\hat{k}$. However, the effect on the standard error of $\hat{L}_\infty$ was less intuitive (Fig. 5). The standard error in this instance decreased with larger ageing variation. The cause of this was the negative bias introduced into the estimate of $\hat{L}_\infty$ (Table 3). The reduced scale of $\hat{L}_\infty$ made the scale of the standard errors smaller, too. However, the coefficient of variation of $\hat{L}_\infty$ was relatively constant between *SD*'s.

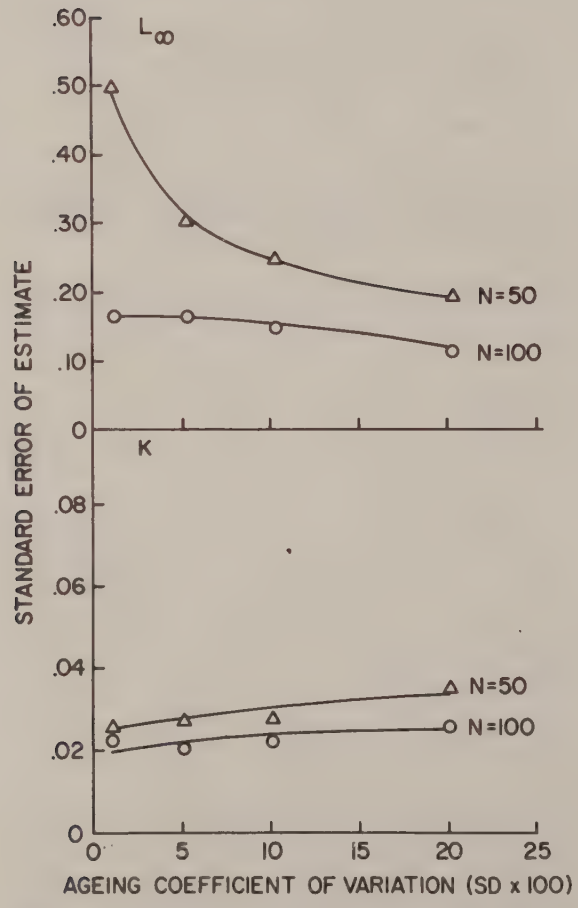

Figure 5.—Simulated effect of the percent random variation in age estimates (SD) on the standard error of the estimates of $\hat{K}$ and $\hat{L}_\infty$ for alternative sample sizes (N = 50, 100). K = 0.1 and L_∞ = 1.0.

It should be noted that rather large biases for both $\hat{k}$ and $\hat{L}_\infty$ were shown when ageing variation increased (Table 3).

Table 3.—Percent bias in estimates of $\hat{k}$ and $\hat{L}_\infty$ derived from 200 simulation runs for alternative random variations in age determination (*SD*). Sample sizes for performing the length age regressions were $N = 50$ and $N = 100$.

Age variation (*SD*)	Percent bias in $\hat{k}$ ($k = 0.1$)	
	$N = 50$	$N = 100$
0.01	4	1
0.05	5	2
0.10	11	9
0.20	35	33
	Percent bias in $\hat{L}_\infty$ ($L_\infty = 1.0$)	
0.01	8	1
0.05	4	1
0.10	0	−2
0.20	−13	−15

Clearly, bias in the estimates of $\hat{k}$ and $\hat{L}_\infty$ was a significant proportion of the error. When this bias was coupled with bias in the age determination (Figs. 6, 7), then the resulting total

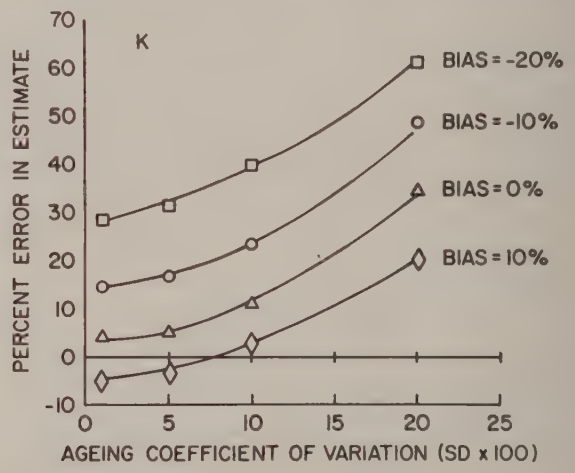

Figure 6.—Simulated effect of the percent random variation in age estimates (SD) on the percent error of the estimate of $\hat{K}$ in relationship to K = 0.1. The effect is shown for alternative percentages of bias (BIAS = −20%, −10%, 0%, 10%) in the age determination method. Sample size for each $\hat{K}$ was N = 50.

bias was less systematic with changes in *SD*. It appears that the estimate of $\hat{L}_\infty$ was relatively less sensitive to these biases in age determination, whereas the least-biased estimates of k seem to have occurred when age was estimated slightly higher and the variation in ageing (*SD*) was < 0.1 (10%). The total error associated with estimates of $\hat{k}$ and $\hat{L}_\infty$ (MSE) are shown in Figures 8 and 9, respectively.

DISCUSSION

The effects of accuracy and precision of age determination on estimates of $\hat{k}$, $\hat{L}_\infty$, and $\hat{Z}$ have been shown in the simulation results. Bias and variation in age estimates led to various

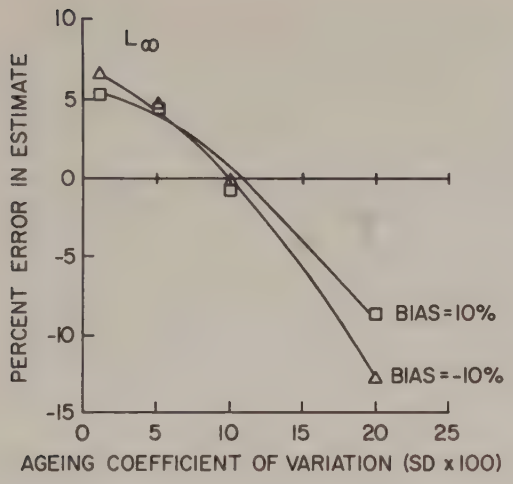

Figure 7.—Simulated effect of the percent random variation in age estimates (SD) on the percent error in the estimate of $\hat{L}_\infty$ in relationship to $L_\infty = 1.0$. The effect is shown for alternative percentages of bias (BIAS = −20%, −10%, 0%, 10%) in the age determination method. Sample size for each $\hat{L}_\infty$ was N = 50.

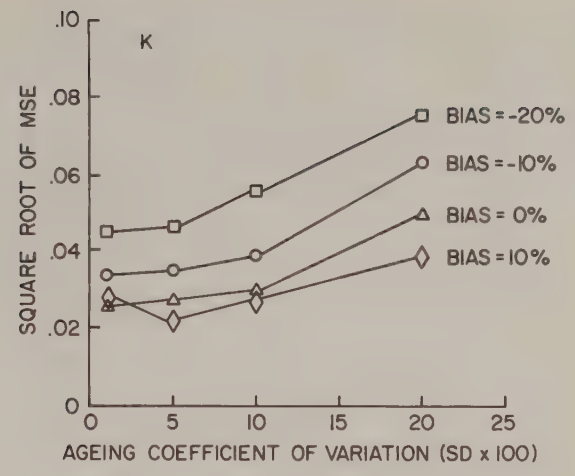

Figure 8.—Simulated effect of the percent random variation in age estimates (SD) on the square root of the mean square error (MSE) of $\hat{K}$ in relation to K = 0.1. The effect is shown for alternative percentages of bias (BIAS = −20%, −10%, 0%, 10%) in the age determination method (BIAS). Sample size for each $\hat{K}$ was N = 50.

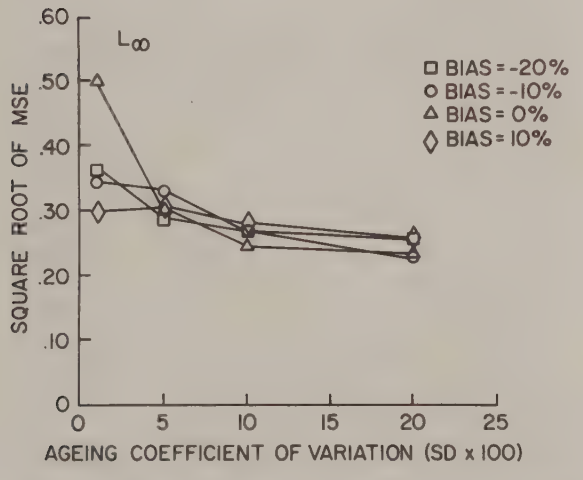

Figure 9.—Simulated effect of the percent random variation in age estimates (SD) on the square root of the mean squared error (MSE) of $\hat{L}_\infty$ in relation to $L_\infty = 1.0$. The effect is shown for alternative percentages of bias (BIAS = −20%, −10%, 0%, 10%) in the age determination method. Sample size for each $\hat{L}_\infty$ was N = 40.

degrees of random and systematic error in estimates of population parameters. However, the mechanisms by which these errors arise have yet to be addressed. Of particular interest are the reasons for which the bias in a population parameter is affected by accuracy of the ageing procedure.

The bias in mortality rate estimation partially resulted from the correction for smali sample sizes. The correction was based upon age being measured without error, which is not normally the case. Apparently, there is a trend toward negative bias as the mortality rate (Z) increases (Table 2).

Another form of bias was introduced by the random normal distribution of estimated age. Since mortality acts throughout the lifetime of the fish, an older fish is less likely to get sampled from the population. Since it was assumed that ageing variation increases with age, the age frequency distribution is more likely to have lower than expected frequencies for older fish than for younger fish. Therefore, the age distribution becomes skewed toward higher frequencies for young fish and the mortality rate is concomitantly overestimated. Bias in the mortality rate becomes accentuated as the percent variation increases.

Similarly, biases in estimation of $\hat{k}$ and $\hat{L}_\infty$ arise when both length and age are measured with error. The age frequency for a given length is more likely to be less than the expected value for older (longer) fish than for younger (shorter) fish due to the increased variation in age determination as the fish becomes older. Therefore, the asymptotic length ($\hat{L}_\infty$) is underestimated and the rate at which the asymptote is approached ($\hat{k}$) is overestimated.

The effect of variation in length-at-age (SL) was not examined rigorously in the simulation model due to limitations of this study. However, SL was changed for a few test cases and the results showed that increasing SL tended to increase the biases in $\hat{k}$ and $\hat{L}_\infty$.

The reason for studying population dynamics is to measure the mortality rate of recruited fish and impacts of incremental changes in the mortality rate. In most cases, a sample of aged fish is not used directly to estimate mortality. Rather, the sample consists of a larger set of length frequencies that are converted to age frequencies using a fitted growth relationship. If $\hat{k}$ is biased high and $\hat{L}_\infty$ is biased low, then the sample catch at young ages is higher than it should be. This would cause overestimation of the mortality rate, as well.

In the process of age determination, we attempt to reduce both the bias and random error in ageing by improving techniques. But the importance of refined ageing techniques should be evaluated in the context of the population rate parameters for which the age estimates are being used. The costs of increased ageing precision may not justify the gain in precision of the growth and mortality parameters.

In many cases, the oceanic pelagic tunas, billfishes, and sharks exhibit relatively low growth and mortality rates compared with other species. The bias that is introduced in their

estimation due to random errors in ageing tends to be eliminated with large sample sizes. This was especially true for the total mortality rate estimated from the ageing data directly. However, when the ageing coefficient of variation was 10% and sample size was 500, the bias in the growth parameter $\hat{k}$ was still 7%. It appears that rather large samples are needed for fitting growth curves of slow-growing fishes with low-mortality rates. If an increase in precision of ageing can only be realized by a less efficient technique, then the sample sizes are likely to suffer. These simulation results indicate that ageing precision of 10% or less is acceptable and that the ageing techniques should be efficient enough to provide a large number of aged fish. Thus, emphasis should be placed on the sample size.

If the ageing technique is biased, then increased ageing precision may actually produce more error in the estimates of the mortality and growth rates. Once again, larger sample sizes tend to reduce both the random and systematic error. However, bias in the ageing technique should be reduced as much as possible. Ageing accuracy is probably more important than precision for most instances. If the bias in ageing is $< 10\%$, then the resulting bias in the mortality and growth rates would probably be dominated by the random error component. It is interesting that with moderate sample sizes, a better estimate of the growth parameter $\hat{k}$ is achieved if ages are overestimated by 10% rather than underestimated by 10%.

Finally, there is likely to be a statistical component as to why various ageing techniques are biased. It may be that the successful ageing of a fish is not biased, i.e., that the ageing method when successfully applied provides an unbiased estimate of age. However, when growth bands cannot be read or when staining procedures do not produce desired results, these fish are often rejected from the sample. Rejections of this type might represent fish from just one side of the probability distribution. Accordingly, one should guard against this eventuality when rejecting fish from ageing samples.

SUMMARY

1) Monte Carlo simulations were performed to estimate mortality and growth parameters as a function of age determination estimated with varying degrees of random and systematic error. The underlying mortality and growth parameters were chosen to mimic the relatively small growth and mortality rates common in the larger oceanic pelagic fishes.
2) The simulations showed that biased rate estimates resulted when there were random errors in ageing, especially for small to moderate sample sizes.
3) Biases in age determination led to biases in the rate estimates. However, in some instances they would tend to be cancelled by the bias introduced by reduced precision in ageing.
4) Methods used for ageing should attempt to reduce bias in the ageing technique to an acceptable level then concentrate on obtaining larger samples. In these simulations, precision in ageing of $< 10\%$ would probably not be worthwhile if the technique made it difficult to process large samples of aged fish. Once precision reaches an acceptable level, research should be focused on developing the efficiency of implementing the technique so that the sample size may be easily increased.

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SUMMARY PAPER

Reduction of Bias Generated by Age-Frequency Estimation Using the von Bertalanffy Growth Equation[1]

NORMAN V. BARTOO[2] and KEITH R. PARKER[3]

INTRODUCTION

Complex population dynamics techniques rely heavily on age structure information. For some species, accurate ageing methods have not been developed. Often the age structure of a fisheries catch (age-frequency) is estimated from sampled length-frequency (Majkowski and Hampton 1983), the age relationship being described by either an age-length key or a growth curve, such as the von Bertalanffy growth curve (Ricker 1958). The growth curve method is used when there are insufficient data to construct an age-length key. But as noted by Kimura (1977) and later demonstrated by Westrheim and Ricker (1978), under conditions of varying year-class strength and substantial overlap of lengths between ages, age-length keys can yield nearly useless estimates of numbers-at-age. Even with bias correction procedures, the construction of a sufficient key can present difficulties.

In this paper we deal specifically with the von Bertalanffy growth equation and the application of stochastic methods to reduce or eliminate biases. However, it should be noted that the method presented here may be applied to any growth equation, as well as to cases where no growth equation has been fitted or where growth is discontinuous, as in crustaceans.

The von Bertalanffy growth equation mathematically models the relationship between age and length, length being the dependent variable (see Equation (1)). As suggested by Gulland (1969), age can be estimated from length by algebraically rearranging the growth equation so that age is the dependent variable (see Equation (2)). Regardless of whether length or age is the dependent variable, the von Bertalanffy relationship is deterministic, i.e., there is a one-to-one correspondence between age and length.

Use of the von Bertalanffy growth equation for age-frequency estimation results in several types of biases (Powers 1983), different from those inherent in age-length keys. In this paper we document these biases and propose a method for their resolution.

AGE-FREQUENCY BIASES

When growth is modeled according to the von Bertalanffy age-length relationship (Brody 1945; Ricker 1958)

$$L_t = L_\infty \, (1\text{-exp}\, [-k(t-t_0)]), \qquad (1)$$

then age, t, can be converted to length:

$$t = t_0 + \ln\,(1 - L_t/L_\infty)/(-k) \qquad (2)$$

where L_t = length at age t
L_∞ = the asymptotic length
k = the rate at which length reaches L_∞, and
t_0 = hypothetical age at which fish would have zero length.

When computing numbers-at-age from Equation (2), estimation bias occurs from several sources. One bias is due to L_∞ being a fitted parameter. Thus, all numbers-at-length greater than L_∞ must either be eliminated or arbitrarily distributed to older ages. Bias also results when lengths approach L_∞ and are mathematically allocated to ages above those attainable by fish within the stock. As lengths (L) approach L_∞, Equation (2) will yield unreasonably old ages (i.e., ages greater than are known to occur).

Additional bias results from the deterministic nature of the von Bertalanffy equation. For example, back calculations of length to age from Equation (2), which are on a one-to-one basis, result in one determined age for any length. In reality, there can be a number of possible ages for any given length, the most probable age-at-length being that with the highest relative contribution of numbers-at-length. Since these back calculations are without probabilistic arguments, the determined age is not necessarily the most probable for the given length.

Back calculations of length to age also result in a mathematical estimation bias due to the substitution of independent and dependent variables in moving from Equation (1) to Equation (2). The degree of bias is likely to be a function of the amount of residual error in estimating length at age in fitting Equation (1). The bias will probably not be consistent between cases and the degree of bias will have to be considered separately for each case. Consequently, biases associated with equation transformation are not specifically dealt with here.

A computer model can demonstrate these biases. For von Bertalanffy parameters L_∞ = 90.0 units, t_0 = 0.0 units, and k = 0.30, predetermined numbers-at-age are assumed normally distributed with a standard deviation equal to 3 units about the von Bertalanffy length-at-age Equation (1), for ages (1) through (10). A length-frequency vector is then generated by: 1) Multiplying the number-at-age times the probability of age occurring within each 0.5 unit length interval, thus generating a vector of

[1]This paper is summarized from research conducted by the authors and appears in *Fishery Bulletin*, Vol. 81, No. 1, p. 91-96.

[2]Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, 8604 La Jolla Shores Drive, La Jolla, CA 92038.

[3]1837 Puterbaugh Street, San Diego, CA 92103.

number-at-length for length intervals between 0 and 100 units for each age, and 2) accumulating numbers-at-length for each length interval over all ages. The numbers-at-age are then deterministically estimated from Equation (2) by accumulating numbers-at-length over the length intervals at age.

The bias from this model is illustrated by input and back-calculated numbers-at-age and their differences, which are listed in columns 2, 3, and 4, respectively, of Table 1. The input numbers-at-age represent a sample age distribution with varying year-class strengths. The differences in column 4 indicate a strong bias which increases with overlap of length distributions at age. The estimated ages of 111 fish were greater than the maximum age, 10. Thirty-five had lengths greater than L_∞ and, consequently, were not classifiable.

Table 1.—Input and estimated numbers-at-age for both the deterministic (column 3) and stochastic (column 5) models, with the input numbers-at-age in column 1. The difference between the input numbers-at-age and the deterministic estimates are given in column 4.

Estimated Age (1)	Numbers at age: Input (2)	Deterministic (3)	Diff. (4)	Stochastic (5)
1	200	199	1	200
2	400	399	1	400
3	800	760	40	800
4	200	267	−67	200
5	600	441	159	600
6	300	378	−78	300
7	400	320	80	400
8	300	258	42	300
9	100	164	−64	100
10	100	68	32	100
>10	—	111	−111	—
Inf.	—	35	−35	—

STOCHASTIC MODEL

With estimated variance of length-at-age, a stochastic model can be built from the von Bertalanffy relationship (or any other growth relation): For any age the probability of a specified length interval is the probability of that interval taken over all length intervals containing that age. Thus, for all ages, a probability matrix ("P"-matrix) of dimension r by c can be computed, where r = the number of rows, or length intervals, and c = the number of columns, or ages, then $P(1,1) = P$ (max. length, min. age). If the number-at-age vector is "a" ($a_{(1)} = a$ (min. age)) and the number-at-length vector is L ($L_{(1)} = L$ (max. length)), then

$$P\,a = L. \tag{3}$$

And as long as $r > c$, then the numbers-at-age vector can be uniquely solved via least-squares:

$$a = (P'P)^{-1}P'L. \tag{4}$$

Applying this stochastic method (Equation (4)) to the previous example, the numbers-at-age generated from the number-at-length vector is given in column 5 of Table 1. Since the probabilities of the P-matrix are the same as those used to generate the number-at-length vector, it is not surprising that the solution yields unbiased results. This computed example illustrates that the stochastic method yields unbiased estimates of age-frequency.

DISCUSSION

Calculation of age from length via the von Bertalanffy growth equation results in several types of bias. The degree of bias is proportional to overlap in lengths-at-age and changes with weak or strong year-classes. When overlap increases with age, age-frequency estimates will generally be more biased for older ages than for younger ages. When overlap occurs, biases will always result, since the numbers-at-length will be allocated to unreasonably old ages. Any numbers-at-length for lengths greater than L_∞ will be undetermined in age estimation, resulting in downward biases for those ages contributing such lengths.

Age estimation biases can be effectively removed by creating a stochastic model based on a matrix of length interval probabilities at age. The probability matrix (P-matrix) is independent of year-class strength and will effectively remove all sources of estimation bias, except that due to random variation in length-frequency estimation. A probability model of the distribution of length-at-age with estimated parameters is necessary for estimating probabilities of length intervals at age for the P-matrix. As long as the von Bertalanffy growth parameters are correct, the stochastic method based on accurate estimates of variance in length-at-age will yield unbiased results.

There may be serious implications to the bias introduced by using the von Bertalanffy equation without bias correction. In fishery management, the overestimation of maximum age by the deterministic von Bertalanffy equation may produce underestimates of mortality rates, which may result in overestimates of population size and recruitment. Further, the deterministic method tends to "fill in" weak year-classes, which results in underestimates of year-class variability and overestimates of recruitment stability. In general, all of these affect accuracy of a stock assessment and contribute to improper advice for fishery management.

Application of the stochastic method shown here to cover other growth equations and situations, such as discontinuous growth, is handled by simply estimating appropriate elements in the P-matrix for each case.

ACKNOWLEDGMENTS

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SUMMARY PAPER

Validation of Age Determination Estimates: The Forgotten Requirement[1]

RICHARD J. BEAMISH and GORDON A. McFARLANE[2]

INTRODUCTION

Like most scientific disciplines, fisheries science has changed rapidly in recent years. In a half dozen decades we have changed our emphasis from observing and describing the biology of fishes to developing large complex ecosystem simulation models. In some cases, our changes were in response to technological advances in other fields, but mainly we were responding to an urgency to understand how fishery resources respond to fishing. We built our understanding and models on the results and conclusions of early studies, the most important of which were the procedures for estimating age of fishes. Unfortunately, we tended to take these procedures for granted and often failed to undertake the validation studies essential to any age determination technique. This failure to assess the accuracy of age determinations has resulted in the collection of extensive data that either cannot be used for the purposes intended, or are used anyway, resulting in significant bias and improper management strategies.

Our review stresses the need for validation. By validation we mean confirming the accuracy of a method of age determination. A clear distinction must be made between the accuracy and precision of age determinations. Precision relates to the reproductibility of age estimates and does not imply accuracy or validity. The various methods of validating age determinations have been summarized by Brothers (1983) and Casselman (1983).

It is the purpose of this brief review to show that the requirement to validate age estimates has been ignored by many fisheries biologists and to suggest appropriate techniques for validating age determinations.

REVIEW OF ATTEMPTS TO VALIDATE AGE DETERMINATION

A survey of 500 age and growth studies published between 1907 and 1980 was undertaken to determine how often and to what extent ages were successfully validated. Validation was considered to be successful when the growth zone considered to be an annulus (see Glossary) was shown to form annually for all age groups in the population. It is important to realize that proving correct interpretation of an annulus for younger fish does not imply validation for older ages. Techniques such as examining the edge of a structure to determine if the zone thought to be the annulus formed once a year, monitoring strong year-classes, or length-frequency analyses are useful for validating the age of younger, faster growing individuals but cannot always be used for the validation of older age groups (Brothers 1983).

Papers included in this review were from journals of a number of countries, with the majority (60%) from North America. The number of papers reviewed had no special significance other than indicating the amount of effort directed to the subject and a conviction that increasing the sample size beyond 500 was unlikely to change any conclusions.

Less than 3% of the studies successfully validated their methods of age determination. Less than 10% used a technique that could validate the method for all age groups in the population, such as mark and recapture or use of known age fish. Sixty-five percent of the studies either mentioned validation or attempted validation. However, most of these studies attempted to show that the annulus was valid for very young fish (the first few years) and then, by extrapolation, concluded that it was valid for all age groups in the population. Six percent of the studies compared age estimates from several structures, but only 1% attempted to resolve any differences that resulted. It was of interest that 20% of the studies implied or stated that, because age estimates were reproducible or precise, they were also accurate.

The applicability of validation techniques in some of the studies in our survey or the relative success of some validation attempts may be subject to different interpretations. However, the overwhelming conclusion remains that fisheries biologists seldom have successfully validated ages despite the clear direction of early workers (Van Oosten 1923, 1929, 1941; Hile 1936) that validation is essential. In fact, an alarming number of studies that use age estimates never consider the possibility that ages may be incorrect.

It is fair to ask if the failure to validate age estimates has made any difference to our understanding of the biology and management of fishes. Perhaps estimating age is so routine that most age estimates are accurate, or that any errors that occur will have little effect on their subsequent use in stock assessment. We believe this is not the case. There are an increasing number of studies that indicate that application of "routine" methods of age determination have resulted in important misunderstandings of the age composition of populations (Beamish and Harvey 1969; Aass 1972; Power 1978; Beamish 1979; Beamish and Chilton 1982; Chilton and Beamish 1982).

[1]Material in this paper was summarized from a manuscript published in Transactions of the American Fisheries Society, Vol. 112:735-743.

[2]Government of Canada, Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

For example, lake trout, *Salvelinus namaycush*; common white suckers, *Catostomus commersoni*; and Pacific ocean perch, *Sebastes alutus*, were thought to be faster growing, shorter lived fishes than now believed. The use of fin rays and otolith sections rather than scales or surface otolith readings (Figs. 1-3) has indicated these fish can be quite old. Furthermore, it is our belief that species-specific problems will be identified once validation of ages becomes routine.

A detailed assessment of the consequences of ageing errors is beyond the scope of this paper (see Powers 1983). However, an obvious concern will be the effect ageing errors have on the various population models. Errors in ageing accuracy will not be random. There may be a bias to producing younger ages that could result in an accumulation of estimates in the vicinity of the age at which the particular technique or interpretation breaks down. Mortality estimates could be overestimated and the importance of strong year-classes can be masked. Other, more subtle features of a population, such as different growth rates among stocks, or the understanding of the ecological importance of longevity (Leaman and Beamish in press) and the mechanisms by which a particular population survives in its environment, may not be detectable.

We stated that successful validation must prove that the fish is not older (or younger) than estimated, as well as showing that the growth zone identified as an annulus forms approximately once a year. To do this, the appearance of the annulus must be studied throughout the life span of the species. At present, this can be accomplished by marking and releasing the fish or by using known age fish. A qualitative or approximate approach is possible using the recent radionucleide technique (Bennett et al. 1982) or by comparing the results of several ageing methods (Beamish 1981).

We recognize that tagging studies are difficult to apply to oceanic pelagic species. However, there have been a number of tagging studies of species such as sharks and tunas where sufficient recoveries have been made to validate an age determination technique. For example, the mark and recapture technique can be adapted for age validation of tuna by removing a few fin rays at the time of marking, for comparison upon recapture, or by applying a "time mark" in some hardpart. A number of compounds have been used for producing a time mark (Yagi et al. 1963; Jensen and Cumming 1967; Yamada 1971; Ellenton and Johnston 1975). However, in our laboratory we have experimented with intramuscular and interperitoneal injections of oxytetracycline (OTC) and have successfully produced time marks in dogfish sharks, *Squalus acanthias*, and other species. The results to date (Fig. 4) have been encouraging and we are currently completing an experiment designed to test the appropriate dosage and type of injection for a marine species. Preliminary results indicate that, depending on the species, a dosage of 25-50 mg/kg of OTC injected into the muscle or gut cavity produces a clear mark in the structure.

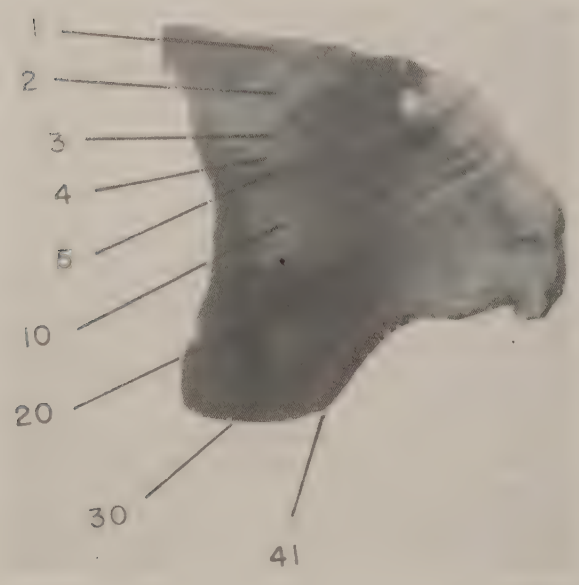

Figure 1.—Portion of an otolith section from a lake trout, indicating that lake trout are relatively old. This fish was part of a study that indicated lake trout and some other species were much older when aged with otolith sections than by examining scales or otolith surfaces (Beamish 1976).

In conclusion, we want to stress that all ages must be validated. We accept that this can be difficult. However, as a minimum, an estimate of accuracy using several structures should be made. If agreement is not obtained, then the consequences of ageing error should be assessed. If these consequences are important, then there is no choice except to undertake validation studies. In some cases, if accuracy cannot be assessed it may be better not to undertake the study.

We encourage biologists to reexamine some long-standing beliefs such as the general applicability of the scale method for age determination (Everhart and Young 1981) or the view that there is a close or isometric relationship between size (length) of fish and size of hardparts throughout the life span of the fish (Lagler 1956).

We believe that fisheries biologists have forgotten to question the validity of age estimates, and in failing to do this they may be misunderstanding the biology and population dynamics of some important commercial and recreational species.

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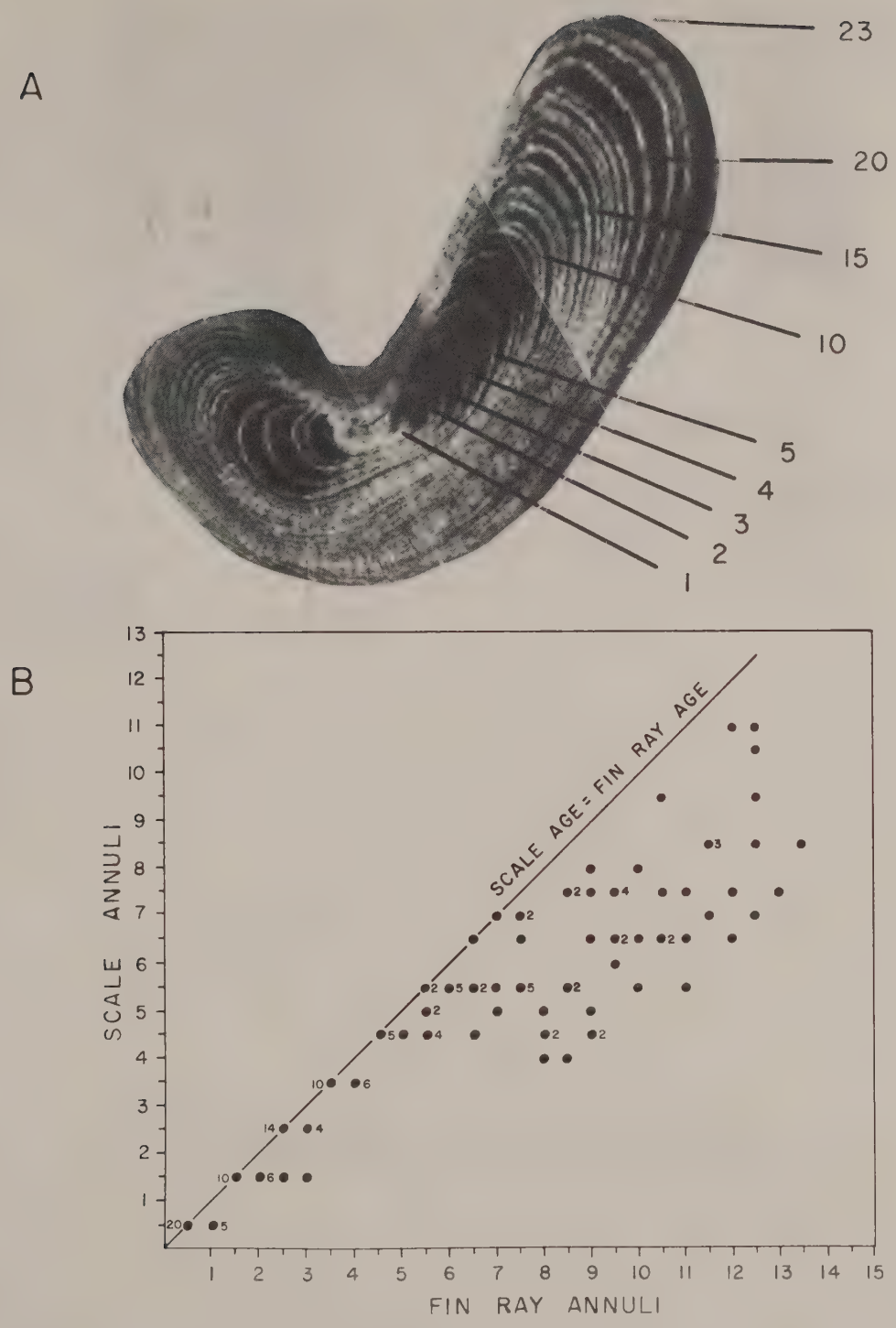

Figure 2.—A) A fin-ray section from a common white sucker (courtesy of B. Paquin, Saskatchewan Research Council). A study by Beamish and Harvey (1969) indicated that the fin-ray method produced more accurate and older ages than the scale method. B) Relation between ages based on scale annuli and ages obtained from fin-ray annuli (Beamish 1973). Points are individual fish except where indicated. Ages determined from sections of fin rays were older after a scale age of about 5 yr.

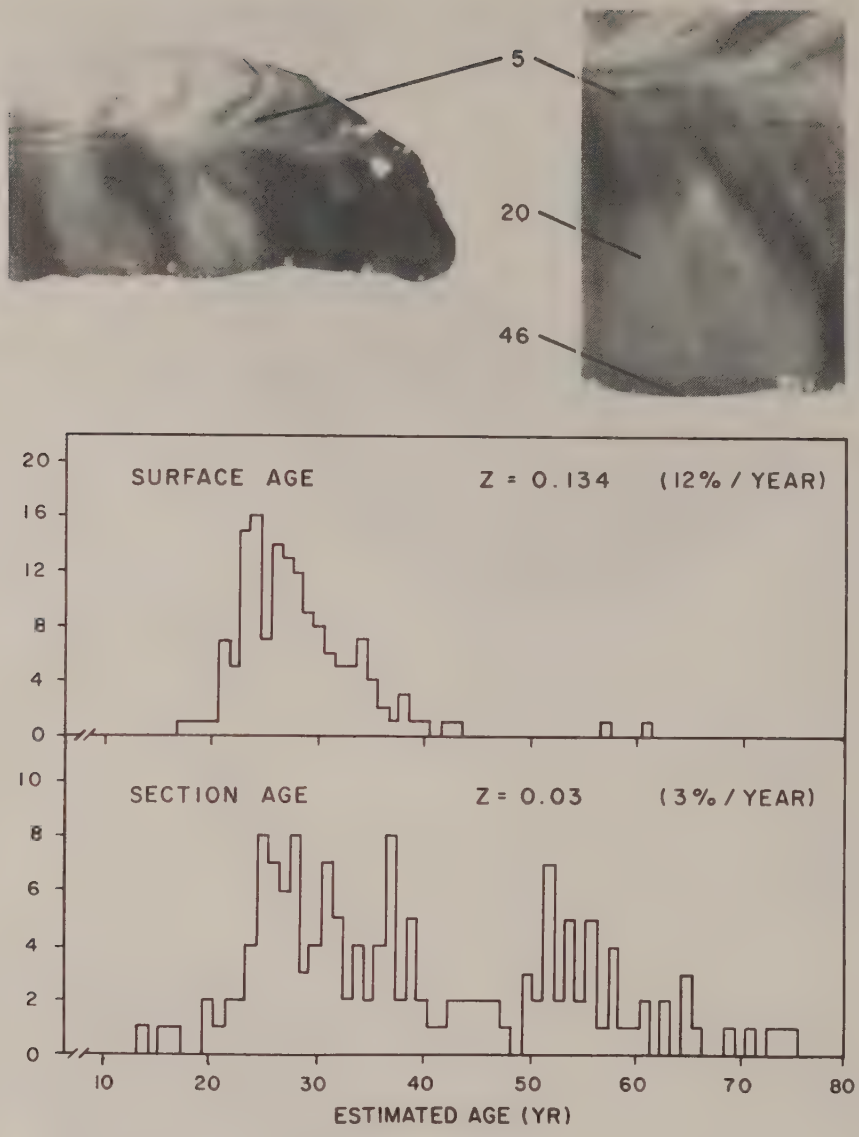

Figure 3. — A) Otolith section from a Pacific ocean perch, indicating that this fish may be very old. A study by Beamish (1979) showed that the age determined from otolith sections was greater than that determined from the otolith surface. B) Comparison of age frequencies from unexploited stock of Pacific ocean perch when aged from the otolith surface and sections. The difference in instantaneous mortality (Z) is also shown (Leaman and Beamish in press).

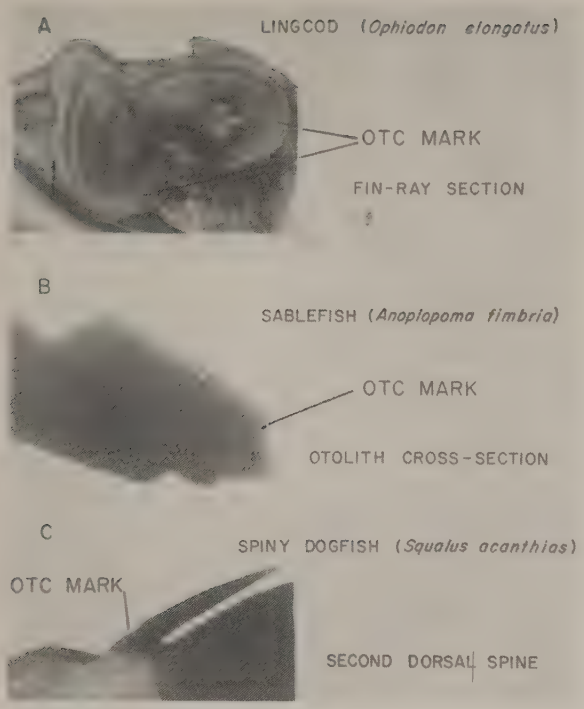

Figure 4.—Structures used for ageing from three species that were tagged and injected with oxytetracycline (OTC). The OTC mark appears yellow under UV light. In all cases, the ageing method was validated by the pattern of growth of the structure during the period the fish was at liberty after tagging and injection. A) Lingcod, *Ophiodon elongatus*, that had been at liberty 2 yr after tagging and injection with OTC. B) Sablefish, *Anoplopoma fimbria*, that had been at liberty 3 yr after tagging and injection with OTC. C) Spiny dogfish, *Squalus acanthias*, that had been at liberty for 10 mo after tagging and injection with OTC.

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Summary of Round Table Discussions on Age Validation

EDWARD B. BROTHERS[1,2]

INTRODUCTION

I would like to find some common ground on terminology. I've made a short list that has about 25 terms that have dual meanings or are inconsistently used by researchers on fish ageing (see Glossary). If we could at least agree on some of these terms, it will certainly improve the consistency of our papers, which are going to be bound together in the same volume. In addition, such a discussion might form a framework for better communication in the future. Hopefully, if we have time later we can go over the list in detail, but I think first we should get to immediate business, which is: What do we mean by validation, and where are we in terms of validating the age and growth of oceanic pelagic fishes?

On the board is a short summary of techniques used in determining the growth rate of a given species of fish (see below).

Outline for Age and Growth Rate Determination of Fishes

I. Tag-recapture and growth in captivity
II. Statistical techniques
  a. length-frequency analysis: Petersen method
  b. modal-progression analysis
III. Anatomical-periodic markers in calcified structures
  a. marginal increment analysis
  b. proportionality of growth and back calculation
  c. comparison of different structures
  d. marking the ageing structure, e.g., tetracycline or "natural" marks
  e. microstructural analysis
  f. comparison with theoretical growth models
  g. correlation with environmental and life history events
  h. establishment of objective criteria; blind readings; reader comparison
IV. Chemical methods

Most are only ancillary validating procedures and are not independent ageing methods. There are three or four basic ways to go about determining the growth rate of a fish, depending upon how one categorizes the approaches. First (I), direct measurements can be made on individuals in a variety of ways. These measurements may result from tagging a fish and recapturing it and looking at its growth rate, or fish may be maintained in the laboratory or an enclosure. Growth rate is determined for individuals, and from that type of information we may extrapolate and say that this is near the expected growth rate in the field, or this is the growth rate of all the fish in the field. Getting good information from tagging studies is a difficult task, as we've seen at this meeting. Aside from these problems, the basic approach described uses direct information about the growth of certain individuals and extrapolates that to the population. The second (II) basic approach is based on a variety of statistical measurements that one can make on population samples. This may include analysis of length frequency histograms at one point in time, or through time if we are looking at modal progressions. There are a variety of other approaches that can also be lumped under the heading of statistical techniques. The third (III) basic approach is the kind of measure that most of us have been involved with here. We might call these anatomical methods, since we are using some kind of temporal marker in hard structures (usually a calcified tissue). Basically, we attempt to assign a unit of time to some kind of cyclical growth discontinuity, i.e., determine a time period over which that structure is formed. We then use these markers to age individual fish. Thus far in the workshop we've spent most of our time talking about the measurement of age and growth from hardparts, and I think we'll probably continue emphasizing that subject in this discussion.

There are various advantages and disadvantages of all of the above approaches. Some are well suited for ageing oceanic pelagic fishes and others can only be applied with great difficulty. I propose that we get a little bit more specific now about the various ways that we can age fish. We've already seen a number of them put into practice during this meeting, e.g., Petersen analyses of length-frequency data (IIa). The method works best in the early ages when fish grow rapidly, and if breeding is somewhat restricted in time. As we know, these qualifiers may or may not apply to pelagic species, and therefore the success of this approach is highly variable. Another related technique is the analysis of modal progressions (IIb). Basically this looks at length frequency histograms over time. If you are fortunate, there are stronger and weaker year classes that can give you markers to follow through time. As I go through these techniques, I want you to think about advantages and disadvantages as they are applied to pelagic fishes. It would be useful if some concensus emerged from our discussion as to which techniques are good and how important they are now or have been and how significant they should be in the future. That is, which should we emphasize and where should the effort go? Which have worked well and which haven't? I think we should try to come up with some recommendations as to where more effort is needed, where we might push for more money to be put into certain types of programs—tagging, tetracycline marking—whatever we feel might be important in the future. If we can speak with a united voice, somebody might listen. We've already briefly mentioned tagging techniques and growth in captivity data and will leave it at that for the moment. Both rely on information on relatively few individuals which is then extrapolated to the population. There are clear difficulties and biases inherent in these methods, particularly for pelagic species.

[1]Chairperson and moderator for the round table discussions on age validation.

[2]Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, N.Y.; present address: 3 Sunset West, Ithaca, NY 14850.

When we discuss anatomical techniques we may be referring to spines or otoliths or rays or scales, those are the primary structures used. In doing anatomical ageing and growth determinations, there are several different aspects to the problem that are quite important. Most significantly, one just can't make an assumption about the temporal significance of a mark without good supporting evidence. In initiating such studies, we have to discriminate the mark, develop the techniques to enhance the structure or substructure in whatever we are looking at, and then determine what the significance is. The final point is: What should we be spending most of our time on? I think we should be validating anatomical techniques by more than one independent or different procedure, but most of us are not doing this. I don't think one validation procedure is sufficient in most cases because usually the data are just not that robust. We also should view structures by a variety of techniques to see if we can still obtain consistent results. This does not constitute validation, but rather is a form of verification, a subject we'll discuss later.

Determining the time and period of band formation is one of the more critical steps in using hardparts for ageing. I am referring to some of the procedures we have seen already, e.g., observing the time of the year when the marginal increment is an opaque zone. This requires serial sampling throughout the year and should include fishes of different sizes, sexes, and ages. The reason for such broad sampling is evident in the literature; fish of various sizes and ages may form seasonal marks at different times in the year, and they may form more than one mark in the year during different parts of the fish's life history. I think we also have to be very careful to use relative measurements when we are doing marginal increment studies, especially if we are dealing with fishes of different sizes, since absolute measurements can be quite misleading and confusing in looking at changes over the yearly cycle. Generally, our catches are not consistent in size structure or age structure throughout the year and this can offer significant bias into marginal increment analysis if absolute measures are used. No matter what particular hard structure we're examining, most everyone agrees that it's difficult to look at, measure, and categorize the margin. Although it is difficult, it is still an important aspect of the validation process and should not be ignored or done carelessly.

Most of us have submitted graphs indicating proportionality of growth of a hard structure and growth of the fish (IIIb). This is important to determine, particularly if one is going to do back calculations of growth. I don't think it comprises a validating step in itself and should not be treated as such. The relationship doesn't have to be linear, it could be of any form, as long as it can be mathematically described and it's consistent amongst individuals. As long as this is true, back calculations of size-at-age are possible. We shouldn't always expect or force the data into a linear relationship because, in fact, many of these structures rarely grow in the same linear fashion throughout the whole life of the fish (see Smith 1983). Comparison of different structures (IIIc) in itself isn't necessarily a validating criterion. It might be a verification procedure and that's something we can talk about later. We will discuss the difference between verification and validation (also see Glossary).

Marking various ageing structures in a fish or having fish of a known age are valuable assets in ageing studies (IIId). Sometimes we have fish that we know are young-of-the-year or only 1 or 2 yr old. We can take known age fish, mark them, and recapture them at a later date to obtain a measure of their growth rate. Ideally, we can also mark the ageing structure to produce a reference point to compare subsequent growth and time elapsed since marking. We've heard of a number of studies in which tetracycline was used: Other chemicals can also be employed. Additionally, there are various kinds of natural marks that one may use, such as those produced by anomolous environmental conditions. This general approach is one of the more definitive ways to validate anatomical ageing studies. The use of natural marks does not necessitate an initial capture and external tagging. Microstructural analysis (IIIe) is something we've heard a lot about already. This is a relatively new technique and it can be of considerable help in determining what is an annual event in the hard structure. In particular, most of the microstructural work has been done on otoliths. In an ideal situation the fish grow continuously throughout the year and also produce a suspected annual mark superimposed upon a daily growth record. We can then test the temporal significance of the longer period time markers by determining whether there is a full year's growth between them. Even if we don't have a continuous 365-d record in the otolith, we can use microstructure data if we know something about the seasonal pattern of growth. There may be changes in growth rate at the beginning and end of a season; perhaps we can correlate these changes with movements of the fish or changes in water temperature that the fish is experiencing. If so, we can still pretty well identify what's likely to be an annual event in the fish, as opposed to what are accessory checks and zones or false annuli. In other words, we may be able to discriminate true annual events from the all too common noise that adds considerable scatter to our age estimates.

Most of us summarize or analyze our ageing data with reference to growth models (IIIf). There are a number of appropriate models, but the von Bertalanffy model seems to be the most popular. Different fish obviously follow different models to different degrees. In addition, different parts of the life cycle may be expressed by different models. Getting or not getting a good fit does not constitute validation. I think that we should look to these models to point out when something is really awry. A poor fit can be a warning signal, but a good fit to von Bertalanffy does not necessarily indicate that you've aged the fish properly.

A common procedure in anatomical ageing studies is an attempt at correlation between the time of formation of seasonal marks and environmental and life history events (IIIg). For example, knowledge of when a growth band is formed on a skeletal hardpart and coincidental information on fish migration or an abrupt temperature change may be reassuring that we're on the right track and there is a reasonable biological or environmental basis for the formation of these marks. Unfortunately, the level of precision typical for such studies on pelagic fish is low: Marks are discriminated in the hard structure, and there's general knowledge that the fish reproduce at about the same time that the marks seem to form. Other events that may produce marks may or may not be considered and the interpretations is muddled; validation is not certain.

A final category of approaches used in anatomical ageing studies involves procedures such as establishing objective criteria, blind readings, and comparison between readers (IIIh). All are very important; however, they don't in themselves offer validation. For example, 30 people may read the same vertebra with the same result, but that doesn't mean that we are

reading the correct age of the fish. These are verification procedures.

We now have a new group of techniques that don't necessarily go with the anatomical ones, though they do use various parts of the fish for analysis. I'm referring to the chemical ageing methods (IV) which are currently being developed and which at least can give us a relative estimate of instantaneous growth rate or some growth rate at a point in time. Very few of them can give us an estimate of absolute age. Perhaps John Casselman can tell us a little bit about that later. I think there are some that can give us an absolute age estimate if the fish are old enough, but primarily the techniques deal with relative growth rate.

What I'd like to do now is open up this session to a general discussion of ageing techniques as they are applied to pelagic fishes. Where do we need more work? I'd like to hear some criticism of the foregoing monologue since it represents a more or less personal view and I'm sure that many of you have different ideas on the subject. I am proposing the following outline as a starting point.

SELECTED STATEMENTS RECORDED DURING DISCUSSIONS

Schwartz—I would like to add one additional item to your list, behavior of the fish. Behavior will determine whether you are going to catch them or not in some cases. For example, hammerheads are best caught on flooding tides and strong currents, not on weak currents or ebbing tides.

Houde—You can further that. Some kinds of behavior are obviously age related. Behavior can tell us something about ageing. For example, we see a salmon in a stream spawning and we know it's a mature fish and we know something about its past history. Ages at which salmon mature have been determined. If we simply observe behavior then we have an estimate of age. Thus, behavior associated with spawning and maturity or being in a particular place where spawning activity might take place tells us something about them. If you are sampling the population, it can quickly tell us something about the age structure of the fish.

Brothers—I can give you a counter example in a similar situation. Precocious Atlantic salmon have been discovered recently that are reproductive but younger than the typical spawners. Changes in life history and sex changes may also give rise to interpretive problems. I guess we haven't come to any definitive conclusions on the question of hermaphroditism in any of the pelagics we've discussed, but in other species this phenomenon can give rise to anomalous results where there are growth spurts after sexual maturity or sex transformation has been achieved. This might be an indication of things to look at in the pelagic species as well.

Houde—I guess I'm not advocating that this is one of the most important things, but I can see where behavior is possibly a useful indicator.

Johnson—One thing that is ignored pretty much in fisheries is chemical behavior in the fish, such as hormone levels and things like that, which we are starting to look at now. Do you think this can give you an indication of when they spawn? This obviously would have some sort of effect on the deposition of calcium or whatever the hardpart structure is made of. There are various chemical techniques that could be looked at that have not been adopted to fisheries research.

Brothers—They've obviously been looked at less in the pelagic species we are dealing with here. These are peculiar beasts compared to the types of fish that most fishery biologists have to deal with. For example, large, highly mobile oceanic pelagic species whose movements transect tropical, as well as temperate, oceans have inherent problems with age determination based on hardpart analysis. In addition, they don't lend themselves well to experimentation. Sonny Gruber (with lemon sharks) has a very nice system and there is some work that could be done in Hawaii on various kinds of scombroids. It's very difficult to do the necessary experimentation or long-term maintenance of fish required for controlled experiments. What really is plausible with these fish and what isn't? Where should we invest more time and energy, and what just isn't likely to be practical?

Johnson—Many techniques can be adapted from human medicine. I'm thinking of one example that deals with various estrogen levels. We could use techniques applied in human medicine, such as radioactivity tagged antiestrogen materials. I know of one paper in the literature on king mackerel where they are examining hormone levels in the blood at the time of spawning. What I am suggesting is that there might be other techniques, especially those dealing with calcium and phosphate in humans and experimental animals, that might be useful for fisheries biologists.

Casselman—I really think that the bottom line here is how do we approach the problem. We have something that's showing some type of physiological record. Anything that affects the metabolism of the organism, such as a behavioral change or a direct physiological effect, is going to be reflected in the hardpart. So, if we want to understand what's going on, then we have to study the physiology of the growth of the fish and of the calcified tissue. I think we are confusing a number of things. The calcified tissue gives us a record and we are trying now to talk about what this record means without going into the basics of the physiology of the growth of the fish. This is really what this is all about. From where I look at it, all our confusion has developed because we have accepted circumstantial evidence. What we've got to really do is separate direct evidence from circumstantial evidence and make sure we know what we're talking about. I think this is where a lot of the ambiguity has developed in terminology (see Glossary). For example, we've looked at a zone and said that it's a fast growth zone. That's incorrect. That's circumstantial evidence. We should describe that zone in terms of direct evidence. Study the chemistry of a zone, e.g., the calcium content of the zone, or if you are looking at it optically, give the optical characteristics of the zone. It has to be direct evidence, and I think that we can solve this problem if we look at those two approaches all the way along. Is this circumstantial or is this direct? When we have something that's direct then we have something to build our science on. That's really important.

Prince—John, that's really a critical point. Talking about circumstantial evidence versus conclusive validation is a big difference and I object to a tone of voice in a manuscript where it seems like there is conclusive validation, when there is not. I think a little adjustment in a few words can make all the difference in the world. Just simply write out what kind of evidence you have. It's critically important to do that.

Casselman—This tone of voice that you are talking about is most important if you are presenting circumstantial evidence and really presenting it as if it's direct evidence. If we sort this

out, I think we can solve our problems.

Prince—There's value in circumstantial evidence.

Casselman—Yes.

Prince—It's very important to record all that we have.

Casselman—But we need to know it's circumstantial and how it's circumstantial.

Brothers—Let's talk about what's circumstantial and what's direct, with respect to the categories of the procedures on the board. If there are other techniques, we can add them. Which techniques give rise to conclusive evidence and which ones are less robust?

Casselman—Almost all the evidence we have is circumstantial. Direct evidence is if you have a beast and it's a fry and you put it in the environment and you pick it up x years, x days later —that's direct evidence.

Brothers—With that one fish?

Casselman—That's right. And if you tag that individual you have direct evidence from the time of release to time of recapture, but you have circumstantial evidence outside that.

Brothers—We're always going to be in the position where we're going to have to extrapolate from individuals to a larger population, so if we stick with that then we are never going to have conclusive or absolute evidence.

Casselman—That's right. We think that somewhere down the line we're going to be able to optically look at a calcified structure and come up with the absolute age. I don't think we're ever going to be able to do that. We may be able to do that chemically or with other techniques, but never optically. You simply have to describe what's in the calcified tissue and hope that you can validate it so you can at least have a relative age. Relative ages are valuable and there are many examples of this in the literature.

Brothers—I don't have any doubt you can do it optically using otolith microstructure. It doesn't work all the time for all fish, but there are a number of examples where it's very clear that it's giving an absolute perfect age.

Casselman—But when we get into the sub-microstructure and the sub-daily problems. . . .

Brothers—There may be other things going on that we may not understand at a lower level. I can't tell you the age of a fish to the minute, but I can sometimes give you the time of day it died.

Casselman—I see another problem. I think we can do things in research that we are never going to be able to accomplish in straight routine age assessment. I think there's a difference here. We are talking about the problems, for example, with the marine stocks in Lowestoff, England, where they age 40,000 fish a year—a routine age assessment. I see problems in transferring this science to a routine type of assessment. We can be very precise but it's going to be very costly and time-consuming.

Houde—I think that in Lowestoff they have formed it into an advanced technology and not a science. In fact, the optical technique that they use there is exact, because certain year classes are well marked in the hardparts. As Ed said, they might not get it to a day or to the week but they certainly know the ages of those tagged cod or herring stocks that they are following. I don't think there is any doubt or hardly any doubt for most of those stocks.

Casselman—They are enumerating zonation in those stocks.

Wild—What does enumerating zonation mean?

Casselman—They are counting and they are coming up with a description of zonation, but it's the same problem as with ocean pelagics and that is, it's difficult to actually mark them so that you have some direct evidence.

Houde—The evidence is indirect but very good because they start out with juvenile indices. They get these fish as young-of-the-year and then sample intensively. There is some possibility of having individual fish aged incorrectly, but as Joe Powers said in his first paper here, when you have a good technique, and I think theirs is good in those northern waters, precision is better increased through larger samples rather than more precise ageing. That's the direction they've gone and I think it's very effective. They don't necessarily manage fish stocks very well, but they do know the age structure of the stock very well.

Brothers—To get back on track here, which anatomical ageing techniques give us good information as to the temporal significance of whatever marks we are counting, and which don't? How does examination of the marginal increment work for pelagic fishes? Conceptually, I feel that marginal increment analysis is a very useful and powerful way to determine when a mark is formed. Does anybody feel that they have a good example where it's really clear that it's working?

Pratt—I worked with the sandbar shark. We came up with some marginal increment data that to me are significant in showing that the majority of growth of the sandbar shark occurs in the summer and points to a winter annulus formation. My senior author is not as confident about marginal increment formation as I am, but I feel that this is a tentative example of a marginal increment study that presently shows promise for determining time of band formation. The problems inherent in this are that annuli or year marks don't always form when they are "supposed" to, or when the investigator needs to see them. It's going to be very difficult to apply because of the narrow banding on some of the larger fish that we really wish to work with. But I offer the sandbar shark as a tentative example of this system working in a rudimentary fashion.

Lawler—It was mentioned when I talked to Jack Casey that the sharks that were held in captivity laid down their rings on an annual basis. He said that there was ocean water being introduced into the aquarium system. The fact that they are laying down these rings in captivity, on a regular basis, just like free-swimming sharks, shows that they are temporally related but not necessarily to growth. It was strange to hear of these fish in captivity, in a relatively closed system, still depositing annual vertebral zones.

Brothers—Other fish in laboratory situations have annual cycles of growth and reproduction even under constant conditions (as best as they can be maintained). That's not unusual, but what might be unusual about oceanic pelagics are the extensive migrations they undergo and the variety of habitats and conditions they experience throughout a year. In fact, it is the unique characteristics of high mobility and extensive migrations over a large geographical range (including tropical and temperate regions) that distinguishes oceanic pelagic fishes from many other species, complicates traditional approaches to resolving age and growth problems, and was the ultimate reason for directing this workshop to address this group of fishes. All individuals in a population may not do this or may do it at different times. This behavioral pattern might make the formation of these zones inconsistent between individuals. If this is so, can we expect to get clear-cut results from marginal increment analysis? Should we pursue it further? Is the difficulty of reading the margin on some of these structures so

great that we are never going to get consistent results, just more technical problems?

Prince—The western Atlantic bluefin tuna is one example where the species ends up off St. Andrews, New Brunswick, sometime in the fall and then in a couple of months is back in the Gulf of Mexico and spawning in the spring. One of the problems we had and the attention we directed in our paper towards annulus formation in the otolith (see Lee et al. 1983) was that when collecting samples throughout the year, small sample sizes in any particular month really weaken the whole approach. More attention should be paid to that. Another thing we noticed as we collected our data from 1975 to 1981 was that these fish don't spawn at the same time every year and when you collect data over that long a span that fact throws all kinds of variability into the system. Bluefin tuna are well documented to spawn in May and June, yet last August a gravid female was caught off Massachusetts. We had the eggs from this fish sent to the Miami Laboratory for documentation. What is a gravid giant bluefin tuna doing off Massachusetts in August? My point is, they don't all do the same thing at the same time. There's a lot of variability in the population and this is reflected in some of the data we've seen. This is very difficult to deal with. I think it greatly weakens our ageing analysis. People are trying to pinpoint the time of annulus formation, yet it may not be very precise in nature. We've got a difficult problem with this in paper after paper.

Pratt—One of the most encouraging things that I saw in this workshop towards that end, Eric, was the work by Richard Radtke with what he calls microincrements. It's the most time-consuming, labor-intensive process I've ever seen—but potentially we could look at the marginal microincrements that we seem to think are daily increments—i.e., to determine the number of marginal microincrements from the annulus to the time of capture.

Brothers—Let's just call them increments for now.

Pratt—Okay. But I'm referring to the scanning electron microscope work, and I don't want it to be confused with other things. We could count back through the days of several clear otoliths, maybe dozens of clear otoliths, and get a statistically significant result to pin down the time of annulus formation. To address another point, you mentioned that this sort of thing would be the one way you could optically validate an ageing method. This is only true if we use a technique that Alex Wild brought to my attention—the work of Joe Tanaka in Tokyo using *Tilapia*. Even microstructure analysis has to be related to real time before you can accept these increments as daily, twice daily, or half daily in occurrence. I know there are ways to do this with larval fish, but if you put a man on the moon with a scanning electron microscope and an otolith, you have got to relate it to real time to validate it. You can't just look at the structure and know what it represents.

Brothers—Exactly! In talking about microstructure and age determination, you've got a whole set of validation procedures which are basically analogous to those used for annual marks. In the way you look at the formation of marginal increments on a yearly basis, you may look at it on a daily basis by sampling fish over different times of day, just the way we would sample them over different times of the year. That's what Tanaka is referring to.

Pratt—The most important thing that we can do before ageing pelagic fishes is to get more methods of direct evidence, or known age methods as Lagler and some of the elementary texts call it. We need these kinds of methods for validation of our counts of rings, annuli, and circuli on skeletal structures.

Casselman—I agree that microstructure appears to give us a specific daily age. But to transfer that to a routine age assessment, you have to go through this relationship of coming up with something on a microstructural scale and then relate it to something on a macrostructural scale. This routine age assessment is a major problem and the things we talk about here may not be necessarily applicable to routine age assessment.

Wild—We have a very similar problem with that in the yellowfin fishery. There is no way that you are going to age part of the catch using daily increments. I look at daily increments as a stepping stone that's very susceptible to validation once they have tetracycline marks. Once you've established structures of this nature that are identifiable, you should look for other types of markers that involve longer periods of time. The objective is to get away from counting edges, because if you are dealing with the commercial catch, then you have to have something that's infinitely faster than that. This is just a stepping stone. If you have to go back to that and use it as an original procedure for ageing, fine, let's start there and then carry on with something that's quicker.

Dean—I totally concur with you. Let me cite an example that was before us this week. We were looking at increments in swordfish and trying to relate them to annulus formation with scanning electron microscopy and light microscopy. Our objective really was to tie our observations with someone else's findings on the same fish using a different structure (anal spines), which are much easier technically to prepare and read. Also, if you want to get into a routine application, spines offer clear advantages. My point is: Do two independent measures on the same fish constitute validation?

Brothers—No. Verification. Agreement between two structures does not validate the structure in question (e.g., the spines) unless the comparative structure is validated. Ideally, the latter procedure is accomplished for the whole life history, not the first year or two. I want to go back to the outline so we all understand what constitutes validation and what doesn't—validation in the sense of determining the temporal significance of a time marker.

Dean—The first step is a move from microstructure analysis to something that can be used routinely. We'll never be able to do the kinds of experiments necessary for true validation with oceanic pelagics (billfishes, sharks, tunas) that many of us have done with cod, toadfish, or other temperate species. We can move one step at a time and accumulate indirect evidence. I think it's going to have to take an accumulation of indirect evidence on pelagics.

Casselman—Definitely, because direct evidence is known age which is seldom attainable under the best circumstances.

Compeán-Jimenez—I work with bluefin tuna in the eastern Atlantic. I can determine with certainty the time of spawning because we know when the fish go into the Mediterranean Sea and we also have a long series of gonad data. The fish go into the Mediterranean Sea in good condition for spawning. In Sicily, fish are also found in spawning condition in the same place year after year. In the fall, one can get good modal progressions for the first two year classes, the only problem is the mixing with western Atlantic tuna. These mixings seem to be fairly minor as Walters stated in his paper. He talks about 15% and this is the high estimate value not the low estimate. Thus, the biology of bluefin tuna is well-known in the eastern At-

lantic, especially in comparison with other tuna. Also, more is known about ageing with a variety of structures. In Europe, we have an acute problem with sampling. This isn't necessarily true for the United States, but in Sicily, for example, if you want one otolith you need to buy the tuna. A 400-kilo fish may cost $400—all for one otolith.

Houde—I agree with Alex Wild that the counting of micro-increments, or whatever we call them, is probably not the routine way to age fish, but don't some of you already use image intensification or computer counting techniques to do some of these kinds of things? What is the possibility of this in the future? Maybe it can be a routine method.

Wild—I think that this has been tried. The problem is that the increments that you plan to look at are subsurface, so you have to expose them (i.e., etching with acid or refocusing optically). And if you can expose them so that they are clear, then you are talking about a technique that could work very well. The problem is you also have to count the increments where they are not exposed very well. The equipment I've used has not been that much help in interpreting what's there, and the human eye is superior because it can integrate materials much better. Where it's difficult to count, you have to guess or estimate. I don't know of anyone that has applied a densitometer technique to count age increments for these reasons.

Brothers—What we are doing is trying to determine what evidence we should be collecting. What evidence really offers us positive proof that the structures we are looking at are formed on an annual or bi-seasonal basis or even a daily basis? Is there some kind of consistent temporal basis to the patterns and can we use them to age our fish?

First on our blackboard list is a method to determine the time and period of formation of a particular mark. It's usually done by sampling fish through time and examining the appearance on the margin of the spine, otolith, scale, or whatever structure it happens to be. It should be done in a fashion where you segregate fish of different sizes and different ages, otherwise complicating variation can be introduced. Furthermore, the relative size of the marginal increment rather than absolute size should be tabulated in order to reduce other sorts of sampling bias. Do you agree that the above is a useful technique? Do you agree that it's satisfactorily executed for pelagic fish? Do you think we should continue working on it? Why haven't we been successful? Sampling problems, microscopic technique, what's our problem?

Casselman—I think it's a combination of things, and I think that the more you look at this, you have to realize that you have to sub-sample, you have to use fish of the same age, preferably, the same calendar year. I think it's powerful if correctly carried out.

Houde—I agree that it's a useful technique. One of the problems with oceanic pelagics is that there's such a protracted season over which they may spawn (although not in every case). There is variation among individual fish. There's a problem. We have to define the distribution for a given population.

Brothers—So a positive result is confirmatory but a negative result doesn't necessarily negate the assumption of an annual mark. Annual marks may occur but they might be formed at different times in different individuals and therefore give a rather shallow seasonal curve.

Houde—We have to understand the distribution. For instance if, simply, there was a normal distribution of frequencies with which an annual mark is formed, you can define the kind of annulus formation in terms of that distribution.

Brothers—Narrowing down the sizes and the ages that you are looking at, as John Casselman said, will certainly help. You undoubtedly reduce some of the variation because fishes of different sizes, ages, sexes, and perhaps stocks, are doing things at slightly different times. So, if we can make relatively discrete samples, partition the analyses, we will certainly reduce the apparent variation associated with time of band formation.

Coffers—A point was brought up yesterday that it's important when dealing with marginal increments to address their distribution rather than the means. You can determine the mean of a number of very small increments and a number of very large increments, indicating that there is formation at a particular time, yet individual values may not be very close to the mean and indicate something completely different.

Brothers—That's why we should look at relative values rather than absolute ones.

Foreman—Sampling is one of the main problems that we should be careful of, especially in highly migratory pelagics such as skipjack, yellowfin, or bluefin tuna. You can introduce incredible error by sampling a fish that's caught by vessels that range over 5 or 6 million square miles of ocean, bringing them back to one central location, and then sampling them and then making generalizations about the age structure or growth rate. We don't know enough about stock boundaries to make an overall assumption for an animal when you don't really understand its stock structure. That's a very dangerous thing to do.

Brothers—So you are saying this is a technique that potentially has value, but because of the biological nature of the fish that we are dealing with there are significant problems. We are not talking about an easily definable population of rainbow trout in a lake. We are talking about a much bigger lake, with several population stocks and so forth. To summarize the discussion thus far, we agree that marginal increment analysis is important though sometimes difficult to execute. To move on to the next subject, could someone explain to me why proportionality measurements are important other than for utility in back calculation? (Also see Smith 1983.)

Pratt—May I back up just for a second while we think about that? Marginal increment interpretation would be greatly facilitated with tag-recapture studies of any sort, as long as they are done accurately and with tetracycline marking.

Brothers—Yes. It would be a more powerful approach. A good point. Would someone like to address proportionality?

McGowan—If you get a linear fit between the growth of the hardpart and length of the fish, it shows that (at whatever rate those increments are being laid down) their growth is isometric to the growth of the fish. So you do have a measure of the growth rate of the fish using the hardpart.

C. L. Smith—It seems to me that this is a case where agreement is very supportive. Disagreement is more critical, though, because if you make back calculations and they don't agree with the observed frequency or the observed modes, then you know that something is wrong. This is an aspect that falsifies your identification of marks as annual marks but doesn't actually prove anything if it does agree with it.

Houde—You said that you couldn't see any use for it except for back calculating. Well, I think it's possibly enough justification.

Brothers—That's important but I get the impression that people look upon this as having some value beyond that and I want to know why.

Houde—But it does tell you something about the way the hardpart grows relative to body growth and you want to know that.

Brothers—Yes, but I can envision a situation where the hardpart grows inconsistently with respect to body growth but it does lay down a beautiful mark every year—sometimes there is a large increment, sometimes a small one. In that case you have a perfectly good structure for ageing, but it's useless for back calculation.

Houde—Okay, that's the negative side of it, but Mike McGowan gave us the positive side if it's isometric—you, in fact, measured the growth rate. It can be very useful.

Stillwell—Is there an alternative? I mean, you have a mark and you would like to be able to relate that mark to something else. Doesn't anyone have an alternative to proportionality measurement and back calculating the diameter of vertebrae?

Brothers—I don't think we need an alternative. It works fine. We can establish relationships and do our calculations. The point I am addressing here is whether proportionality determination validates the usefulness of the markers in terms of our being able to use them to age fish.

Prince—This is an important point. Eighty percent of the papers submitted for publication in our proceedings had proportionality analysis included. I talked with Frank Schwartz about this topic. Apparently, fulfilling the proportionality assumption is not necessarily an important criterion for determining average age, but it is important in terms of back calculating previous growth history. Concepts of age and growth are so closely aligned that they are almost always referred to in the same context. However, they are distinctly different ideas that should be kept separate, particularly when it pertains to proportionality measurements. It should be clearly recognized that when you don't have a good relationship between size of fish and size of hardpart, the utility for back calculation is suspect at that point. For example, spines on swordfish seem to have a good relationship (see Berkeley and Houde 1983), but the otoliths from these same fish don't seem to show isometric growth (see Wilson and Dean 1983). In this case, let's not try to back calculate previous growth history using otoliths, let's stick with a better structure.

Wild—That was very important in the yellowfin study that I did. I examined several different variables and all were correlated with each other and fork length and weight. So what? They're all measures of growth; they're not necessarily measures of time, which is what I'm interested in. It strikes me that the important variable or relationship to look for is between time and some other growth characteristic that has the minimal variance for prediction. It's unimportant to me at this point that the length of the otoliths is related to fork length. They're both measures of growth but have nothing to do with time.

Casselman—That's a very good point. We have a whole other problem here when we start talking about growth. When we talk about age and age validation, that's one thing, there's a time parameter. But when we start talking about growth, then all the work that I've done with tetracycline indicates that the more you do with it, the more you suspect your growth work. You're describing the growth of a particular structure and then you're trying to relate that to body growth or some other parameter. The back calculation problem is another problem and I prefer now to just address the ageing problem.

Brothers—Let's move on to the next point. The comparison of the different structures, scales vs. otoliths vs. spines vs. vertebrae: How important is this in ageing studies? What does it tell us?

Compeán-Jimenez—This point is very important for practical considerations. We should sample several structures since some may work better on young fish, while others may be more useful for older fish.

Brothers—Yes, there is a practical aspect to it. You may not be able to use the spine but the vertebrae may be very good. We should sample a variety of structures. But do comparisons help us in terms of validating annual or seasonal marks?

Foreman—We may look at what we call daily increments and we may get a good validation of a daily increment series on an otolith and then go back and do a whole count on an otolith. If we're convinced that we have daily periodicity for the whole otolith, it seems to me it's pretty easy then to count the number of rings on a spine or vertebra and compare the two.

Brothers—If you have one validated structure or time marker then you can look for agreement with other structures. Thus, it takes one validated structure to validate another.

Foreman—That makes sense to me.

Brothers—But does agreement between two non-validated techniques tell you anything?

Foreman—No. If you haven't validated anything it won't work. All you have are two verified, non-validated structures.

Prince—That's the point of the whole thing.

Unidentified—Comparing two structures is still important. I've noticed this especially in the bluefin papers. Non-agreement between two structures can tell you a lot. It seems that it can point the way to further research. The fact that you're not getting the same age should raise suspicions. It's very important to look at multiple structures.

Brothers—Good point, also.

Compeán-Jimenez—We should also remember that tetracycline injections mark several structures at once and therefore we can potentially compare and validate several ageing structures at once.

Prince—My interpretation is that agreement between two different unvalidated hardparts is verification, i.e., that both hardparts are showing the same number of marks and this indicates that perhaps they were both responding to some environmental stimulus at the same time. But that's verification, not validation. That's the distinction I've made between validation and verification.

Brothers—To summarize thus far, we agreed that IIIa may constitute validation, but IIIb does not. Procedure IIIc only works if one structure is already validated, so let's go to IIId, marking ageing structures with various chemical tags or depending upon some sort of natural tag. For example, we may identify a particularly good year that leaves a characteristic mark in an otolith, as has been seen in temperate fish populations. We then sample over a sequence of years and count from the anomalous natural mark to the margin, comparing the number of presumed annual marks to the known elapsed time. This is basically what we do when chemically tagging an ageing structure. How important is this to validation?

Foreman—Some medical literature on tetracycline indicates this chemical inhibits bone mineralization and alters growth. In some cases, body growth would increase (I believe this involved chick embryos). Marking procedures may cause a slight

bias in growth. It's something we should be aware of more than anything else.

Prince—Al, you've injected a lot of fish, is that a significant problem?

Wild—I think basically what the literature says is that it can accelerate growth but it can inhibit calcium deposition. And that's pretty critical if we are going to be using this material as a marker.

Foreman—I think that's dosage related, though.

Brothers—I haven't seen any evidence of that phenomenon. Has anybody seen any evidence of that in their work?

Casselman—In the chick work, the dosage was quite a bit higher than we are using. If there is an inhibition of calcification, a very distinct mark should be produced at the point of tetracycline deposition. I've done some probe analysis across my tetracycline zones and I couldn't find any difference or any decrease in calcium content. The effect referred to in chicks just hasn't shown up.

Foreman—I think someone showed slides with a very wide tetracycline mark that was being deposited over months at a time.

Casselman—Well, you could see a wide mark, depending upon dosage rate.

Prince—It was in shark centra shown by Gruber. I guess he used a very large dosage. The entire centrum was yellow, but you could see that it still had a very distinct mark at the top. Is that what you're referring to?

Foreman—Yes. I was thinking that maybe that has some bearing on the question.

Casselman—Well, you get this over-fluorescence because you have more than just uni-dimensional growth. It's really critical when you start working with tetracycline to get the right dosage rate so that you can see exactly where the mark is. The usefulness of the technique depends on our ability to be precise in locating the mark.

Stout—In reference to the shark centrum Terry mentioned, we just wanted to show that it was not the same as the others. We were pointing out an anomaly in the system. There is no apparent reason for that, it wasn't because it was a short-term effect and hadn't as yet been incorporated. It just didn't follow the pattern others had. The band was just as narrow, just as discrete, and from the visual analysis we gave it on the surface and in various cross sections, it didn't look any different.

Brothers—I think we agree on the utility of the technique. What do we want to say about the feasibility? What problems have we encountered in working on pelagic fish on the high seas, tag return problems, and so forth? Should we put a lot more emphasis into this? Should we ask for more support?

Wild—I think it's important to look for more concentrated solution forms that we can use. In injecting yellowfin, for instance, if you try to put in more than 2 cc's of 100 oxytetracycline, it tends to back up and flow out of the needle pore. All of the otoliths that have been returned from injected fish do have a mark, so there's never been a problem. But I talked to Eric Prince, for instance, and what do you do when you are trying to deal with a large bluefin that may take a quarter of a gallon? What we need is some more concentrated material that you could apply under pressure, possibly several different kinds of chemicals.

Cayré—In our preliminary results from tetracycline injection, we noted that we don't have an identifiable annual mark produced each year. The mark may appear in a different form or number in different years. We have to do more work on this.

Brothers—So in that case, you may never be able to use any particular mark as an index to age individual fish, but at least you'll get growth rates on those fish. Okay, let's move on to microstructure, which we've already discussed to some degree. Are there any other comments that we want to make about it? There's clear utility in the technique in helping to validate other kinds of longer periodicity marks. For juveniles and larvae, it can be easily applied to directly ageing individuals. There are, of course, problems with it. It's time and energy intensive and does require its own form of validation.

Foreman—For microstructure studies, we should probably start looking for a kind of cross-referencing of the different hardparts. For example, we should look for coincidence of marks and back-calculated fish size for different ageing structures.

Brothers—Is what you are saying akin to validating one structure with another?

Foreman—Yes. If we could get a good relationship between otoliths and centra, for example, we might be able to really pin down annulus formation by back calculating one structure on the other.

Prince—Microstructure studies have mixed validation and verification problems. In the larvae, there are good validation techniques. If you raise larvae in the laboratory and count presumed daily increments you can validate conclusively. When you get to mature fish where major bands are unvalidated and you count the minor bands in between them, this is not necessarily a concrete validating technique. It comes more into the category of verification, and it's not that conclusive.

Brothers—Yes. If you are using otolith microstructure and if you are saying something about information you can get from daily growth units or daily growth increments, then you've got to demonstrate or validate that those are in fact formed with a diel periodicity.

Prince—The one exception is the work by Alex Wild where he had tetracycline marks and counted the daily increments on the margin of mature fish. You can do that, but without some kind of a chemical mark, I don't see how one can validate daily growth increments in large fish.

Dean—I concur, but I think we're also going to be facing the fact that we are going to have to accept a certain amount of transfer of information. I don't really feel that there is that much difference between a yellowfin and a bluefin, biologically. If Alex can directly demonstrate daily increments in yellowfin and we can see the same microstructure leading to annulus formation in bluefin, I don't know that we necessarily have to have tetracycline labeling of bluefin. So I think we are going to have to ultimately accept a certain transfer of information between species.

Wild—I disagree with that. When we worked with the skipjack, results showed that, at least over a period of time during the growth of skipjack, one increment was not equal to a day. I need to find out at what time during the growth of a fish increments stop equaling a day. I think there is a reasonable amount of evidence to indicate that during the larval or juvenile stages that one increment does equal a day. After these early stages, this generalization is much less certain.

Johnson—I'm not too familiar with the intricacies of the microstructure analysis, but I believe that one paper mentions something about a possible mark that might be put on a lunar

basis, something done in Hawaii. Mentioning the fact that increments might not be daily marks in older fish comes back to knowing about the life history of the fish and what it responds to. There may be some kind of photoperiod situation that's not really 24 h. There may be spawning every 28 h, and that's why you could be getting 340 marks a year instead of 365.

Cailliet—Would you include in this category the histological characteristics of calcified bands in elasmobranch vertebrae, or would you include that in the first category of determining the time and period of formation? I would like to include it in the microstructure.

Brothers—I agree and believe we should move further in this direction of anatomical and chemical studies. Jack Casey has started to do this. Certainly it will help to refine our understanding of what those bands really represent. It's not quite the same as the SEM studies but it will tell you whether you have large cells, small cells, dense concentrations of calcium, and so on. It can be carried out at the edge of the centrum in samples taken over time, but it has a greater potential value.

Microstructure might also be broadened to include chemical analyses and studying the metabolism of calcium and other constituents. From these sorts of data we should be better prepared to interpret the significance. Let's go on to comparison with growth models (IIIf). What does a fit or non-fit to a von Bertalanffy or any other model tell us about verification or validation?

Wild—It doesn't tell you anything about validation.

Brothers—Correlation with life history and environmental events (IIIg): Of what utility is this sort of information? Is it simply reassuring to know that the marks we're using seem to relate to the biology of fish? Is that all we can say about it?

Compeán-Jimenez—There is a problem with this approach since the life history of the bluefin tuna is poorly known. Furthermore, there is a problem with stock definition.

Brothers—Is it worth pursuing? Occasionally we do get a correlation, so what?

Prince—I think looking for correlation with events and possible causes is significant, but I'm not sure it's directly related to conclusive validation.

Johnson—Consider a bluefin tuna or some other species that has a very discrete spawning period in which it spawns during a couple of weeks of the year. Maybe it has to have certain light and temperature conditions. A time mark may be produced by such an event.

Brothers—How do you know that it puts down a mark at that time?

Johnson—Well if you can show it, that's what I'm saying. You have to know the life history or you're back to marginal analysis again. If you don't know the life history of a species, then you don't know what you've got.

Brothers—You may know the life history of the species but how do you know that a mark is formed at a particular time and only at that time?

Johnson—By virtue of your marginal analysis.

Brothers—Okay, so we're back to that again.

Smith—In Brothers' work, he was able to correlate the appearance of anomalous daily increments in freshwater stream fish during unusual weather conditions. That seems to me a perfectly valid way of correlating this. It's essentially a natural tetracycline event here and I think it's a very important aspect to validate these marks.

Brothers—Yes, I suppose that goes under the category of utilizing natural marks. Objective criteria, blind readings, comparison between readers, etc. (IIIh): These obviously help in verification. Do they do anything in terms of validation?

Wilson—It's a helpful analysis. It does add credibility to what you're looking at. Sometimes there are great variations in counts conducted by only one person. On the other hand, there may be relatively little variation by one reader, but another may have a completely different interpretation.

Foreman—What that does is give you reproducibility. It may not validate any temporal significance, but it sure increases the confidence you have in your results.

Casselman—What we are doing here is measuring consistency, and that's the way I'd like to look at it, regardless of whether we're doing it between interpreters or within interpreters or within a study or between studies.

MacLellan—Verification vs. validation is precision vs. accuracy (see Glossary). What we are after in determining whether an ageing technique works is accuracy.

McGowan—I would like to comment about the von Bertalanffy fit we saw yesterday with the shark data. The ages were unvalidated and the von Bertalanffy curves didn't seem to quite reach L_∞. From a practical point of view, it might be better to accept those curves as being based on the best available current data, whereas the L_∞ derived from some rogue shark that was caught before there was actually a fishery may be an unrealistic value. You might want to consider it even though the fits don't seem too good. They may actually be appropriate for the population that exists now.

Cailliet—It seems to me that goes back to procedure IIIf concerning growth rate determination and comparison with a theoretical growth model. I agree with you that there's a lot of individual variation. The largest reported size doesn't necessarily have to correlate with L_∞. The von Bertalanffy growth model may not fit the curve the best. We just used it as a rough approximation to see if we're in the ball park. I think it's useful in that instance. It certainly isn't a very powerful validation procedure.

McGowan—Right, it doesn't validate it, but in terms of modeling the growth, it may actually be better than it looks using that other L_∞.

Foreman—I think it gives some insight. Because, like the data that I'm looking at, one to three age classes show up in bluefin in the Pacific—that's all I can sample on bluefin and when I calculate a von Bertalanffy curve, it's based on the fast growth period of their life. I get an L_∞ of about 750 cm. I know this is an unrealistic L_∞ since the fish do not grow to anywhere near that size in the Pacific. It's a good tool, but it's not validating anything.

Cailliet—Yes. I think this goes back to Ed Houde's comment yesterday. With a few points and the equations you can complete a von Bertalanffy fit and still not know what the valid time frame is.

Hurley—I agree with Terry Foreman. It's a descriptive technique more than anything else. The only other thing that it might do, though this isn't directly related to ageing, is that sometimes fitting some sort of model gives managers a tool they can use in stock assessment.

Houde—I want to add to Peter's comment. We're all trying to age fish for some useful purpose. Most stock assessments depend on either cohort analysis or yield per recruit analysis and in both cases the von Bertalanffy fit seems to be the stan-

dard way to get a growth curve and determine the age structure of the population. In the yield per recruit plot, both the k's and the L_∞'s are worked right into the model.

An additional comment I have concerns what I feel has been a surprisingly underemphasized category, i.e., the use of long-term tag-recaptured fish as a source of hardparts and age and growth validation. As we saw in at least one paper (Lee et al. 1983), this can be a valuable source of information and more effort should be expended in this direction.

Brothers—At this point, we have to draw our discussion to an end. I'll let Eric Prince have the last word.

Prince—One recommendation that I think we can all agree on now is that we need to make a greater effort to obtain a variety of hardparts from tag-recaptured oceanic pelagic fishes. Improving rewards for tag-recaptured tunas, billfishes, and sharks is one approach which may eventually provide at least a partial answer to the validation questions we have discussed today.

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Summary of Round Table Discussions on Back Calculation

C. LAVETT SMITH[1,2]

BACK CALCULATION

Back calculation, defined as the process of determining how large an individual fish was at some previous age, is done by comparing a standard dimension of some skeletal hardpart (i.e., scales, vertebrae, otoliths, or spines) with a standardized measure of the overall size of the animal. If there are growth zones on the hardpart that can be related to a time scale, back calculation will make it possible to trace the growth history of that individual fish.

Back calculation is a powerful tool for the fishery resource manager. By monitoring average growth rates of various age classes (cohorts) in the population, it is possible to identify fast- and slow-growing seasons or years. This information can be used to establish correlations with environmental factors and ultimately provide the baseline data for constructing predictive growth models. The ability to age fish accurately permits assessment of reproductive and recruitment success and calculation of mortality rates and growth parameters. Back calculation greatly increases the amount of information that can be derived from each specimen and provides a means of monitoring biomass production of each cohort.

BASIC ASSUMPTIONS

One of the most obvious assumptions in making back calculations of an animal's past growth history is that there is a predictable and unchanging relationship between the size of the skeletal hardpart and the size of the animal. The hardpart is usually measured as the linear distance between a central point (i.e., focus, nucleus, or core—see Glossary) and the periphery of the structure. The length of the fish is normally taken as a standardized measurement (standard, fork, or total length), although, under some circumstances, other measurements may be substituted. For example, sailfish, *Istiophorus platypterus*, taken by sportsmen often have the rostrum removed before they are examined by biologists (Hedgepeth and Jolley 1983). In this case, an alternate measure of length (distance from the center of the pupil to the fork of the tail) was adopted, making it possible to use many more of the specimens from the sportfish catch.

Previous research has often demonstrated that the relationship between the size of a hardpart and the size of many species of fish can be adequately represented by a straight line that may or may not pass through the origin. In other cases, the relationship is better expressed by a curvilinear expression (Lee et al. 1983). Therefore, there is no justification for routinely assuming that this relationship can be adequately represented by a straight line and the relationship should probably be checked empirically for each population. In addition, numerous examples in this volume (Lee et al. 1983; Wilson and Dean 1983; Radtke 1983) have demonstrated that a strong relationship between certain hardparts (particularly otoliths) and the size of fish does not always exist. In these cases, back calculations should not be attempted because violation of this assumption renders the results dubious.

Another assumption involving the use of back calculations is that growth zones on hardparts should be related to time intervals. In temperate areas where there are well-defined seasons, winter cessation of growth can produce marks on hardparts that can usually be distinguished from marks that are a result of spawning, temporary starvation, short-term environmental perturbations, or other causes. True annual marks provide a time scale against which size can be plotted to produce a growth curve. To some extent, agreement between back-calculated lengths for each year mark (annulus, see Glossary) and the observed size of the fish from the same cohort at the end of successive growing seasons serve as an indirect means to validate growth zones as true year marks. However, if there is some variation in the time of formation of the annual mark, this can introduce a sizeable error and this problem should be addressed.

ADDITIONAL CONSIDERATIONS

Once it has been established that the observed marks are produced at some reasonably consistent time interval (these need not be yearly, so long as they are consistent), the distances between the marks, or the successive body lengths calculated from these distances, can be used for fitting growth curves. It must be emphasized, however, that a calculated growth curve is only an approximation of the actual growth history, which consists of alternating periods of slow and rapid growth. It is this variation in growth rate that produces the translucent (fast growth) and opaque zones (slow growth, see Glossary) that constitute the growth marks. For some purposes, it might be desirable to obtain a truer representation of the growth pattern. For example, accurate models might make it possible to control fishing effort so that the harvest would be reduced during the periods of rapid growth, thus allowing more individuals of a particular cohort to survive until they reached a length plateau.

Since growth rate depends on many factors, not all of which are thoroughly understood, periods of rapid growth can best be determined empirically rather than through theoretical models.

Conspicuously few attempts at back calculating length of oceanic pelagic fishes were presented at the workshop. Most

[1]Chairperson and moderator for the round table discussions on back calculation.
[2]American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024.

growth curves have been based on observed lengths of fish whose age could be estimated from hardparts. From this I conclude that most of the workshop participants do not believe that the basic requirements for accurate back calculations have been met, or that many of us have just not reached this stage of analysis. Presumably we all agree that the first problem lies with the time axis. There are still difficulties with the validation of growth marks as annual events (see Brothers 1983).

Merely demonstrating that one pair of opaque and translucent zones are formed each 12 mo will not be sufficient for accurate back calculation unless it can be determined exactly when in the year the transition from opaque to translucent occurs. It now appears that the assessment of daily increments on otoliths may help solve this problem or at least reduce it to manageable proportions. Obviously, the counting of daily increments requires too much time and skill to use it routinely, unless it becomes possible to automate this process, using scanning electron microscopy and microprobes. If this could be accomplished, then back calculations could be developed into a very precise technique.

If the time frame problem can be resolved, then it may be beneficial to reexamine the assumption that hardparts, once formed, do not change dimensions. It appears for some species that shrinkage of the central portion of scales with increasing age might account for the Rosa Lee phenomenon (tendency for back-calculated lengths at a given age to be smaller, the older the fish), so commonly encountered when making back calculations from scales. The problem is perhaps less likely to occur with otoliths and other hardparts, but it certainly should be considered and tested if possible.

During our discussion, there have been several suggestions that standardizing our technique would be advantageous and allow results between studies to be more readily comparable. It is true that there are several areas in which standardization of back calculation techniques will ultimately be desirable. For example: What measurements do we use? How do we measure marginal increments? How do we deal with hardparts in which the center of the structure is reabsorbed or early increments obscured due to vascularized tissue? What mathematical treatments are we to use to describe growth histories? What will be acceptable as adequate validation of marks? (See Brothers 1983.) How do we deal with a situation when a good relationship between size of fish and size of hardpart is not evident?

Given the present state of the art, it seems undesirable to attempt such standardization at this time. We are still very much in an exploratory research phase, and because there are no universally accepted techniques, workers have had to develop their own ideas, and they have been free to ask, "What observations will be most informative?" If there were set standards, there would be a temptation to try to bend the data to meet standards that in some cases may be quite inappropriate. It is my opinion (and that of many workshop participants) we are better off without guidelines at this stage, provided we keep informed as to what other research workers are finding. At this time our most crucial needs are closer communication and flexibility rather than rigid standards.

SELECTED STATEMENTS RECORDED DURING DISCUSSIONS

Foreman—About a year ago, I think there was a paper that dealt with standardization. It made the point that no one could tell how the calculations were made unless the technique is spelled out in the paper. Perhaps we could adapt something like the procedures suggested by F. W. Tesch in the IBP Handbook [Methods for Assessment of Fish Production in Fresh Waters, W. E. Ricker (editor)]. Is that a good reference, John?

Casselman—It's pretty acceptable. I don't like back calculation in general. It's a completely independent subject from age determination and has its own set of problems (many unresolved).

Casselman—If we think we have problems in age assessment, we haven't seen anything until we start looking at back calculations. I'll give just one little example of this. I found this really confusing when I was trying to sort out my problems in looking at the growth of various structures in relation to the body. I would go to the literature and would find the statement that the fish grew at a certain rate during a certain period of time in the year. I looked at this, and it didn't make sense in the model I was building in my own mind. Low and behold, the growth rate that the person was talking about, in terms of body growth, was in fact something they'd constructed from the scales. So they were really talking about scale growth and just automatically calling it body growth. I think the first important thing is that when you're working with the growth of a structure, you're describing the growth of that structure—you're not necessarily describing exactly what is going on in the body of that fish or in another structure of that fish at that time. I think you have got to make sure that you explain that.

Foreman—I think that in the future, someone, or perhaps a group of people, should do a review paper on back calculations. I went through some bluefin literature and over and over I saw that the authors say they use the scale method. Good grief! What's the scale method?

Casselman—I think the next step (beyond the scale method) is the use of tetracycline. When you come back with that marked tissue that we can relate directly to a time scale, then we can talk about growth.

Unknown—There's a growing amount of statistical literature on the inappropriateness of using model one regression techniques where model two techniques should be used. There's error in measuring lengths and weights of fish and these types of experimental errors violate model one assumptions. It has been suggested that functional regressions are appropriate in most fishery work. Right now, however, there is no valid way to compare functional regressions.

Wild—I think we should ask how many people believe what Ricker had to say concerning the subject. I don't believe it's significant.

Unknown—I know that Dr. Carlander doesn't particularly agree with Ricker's conclusions because it depends on how well your data are correlated, whether you use functional or regular regression.

Johnson—We've run into problems with port samplers. I think the definitions of things like total length are variable between studies. I know one slide that we were shown this week was based on total lengths taken after the tail was squeezed together. Where I come from you don't squeeze the tail. I don't know who's right but it can make a big difference. I did a paper on yellowtail snappers and I found that some port samplers were squeezing the tail and some weren't. Use of fork length and total length can also be very confusing when there is a 15 to 20% difference between them.

C. L. Smith—It makes a significant difference and it's obvious that somewhere we've got to get together and standardize these measurements. I do think it's healthy that we're addressing these things, and I believe we're going to have to ask a lot more of these questions. The statistical questions are definitely going to have to be addressed by someone with a good mathematical background. However, I think it's a concensus of this group that we simply aren't ready to settle down to any standardization of back calculations. Back calculation will continue to be an important tool and will probably become even more useful when it's refined, but for oceanic pelagic fishes, we simply aren't ready to make a set of recommendations here.

Crow—I have a question. How many people have actually compared the measurements of the second year growth mark on, say, a 3-yr-old fish, to the second year growth mark on a fish that is 10 or 12 yr old to see if there is any positional change? I think that is important.

Martin—I did, not knowing what the growth bands I was counting represented. I made the assumption that they were annual and I found a slight Rosa Lee phenomenon. I had 23 age classes. I felt that my data on the upper age classes were rather limited. I felt that back calculation worked, although I calculated some correction factors and did a regression analysis. It seems that back calculation will be useful once we figure out what circuli we should count and what time period they represent.

MacLellan—It is interesting to compare growth curves generated from back-calculated sizes with those derived from the size at time of capture. I don't know how useful this is, but it is interesting.

C. L. Smith—I would think that if there is agreement, it doesn't necessarily mean that the calculations have been verified. If, though, there is strong disagreement, it is a sign that something is wrong and we need to get busy and find out why.

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TUNAS

Age and Growth of Young-of-the-Year Bluefin Tuna, *Thunnus thynnus*, from Otolith Microstructure

EDWARD B. BROTHERS,[1] ERIC D. PRINCE,[2] and DENNIS W. LEE[2]

ABSTRACT

Video-enhanced light microscopy was used to examine the microstructure of otoliths (sagittae and lapilli) of 369 bluefin tuna larvae, *Thunnus thynnus*. The larvae, ranging from 4 mm notochord length (NL) to 9 mm standard length (SL), were collected by neuston and conical plankton nets near Miami, Florida, in May and June 1981. The otolith age distribution (total number of presumed daily growth units) showed a pronounced peak at 8 d (50% of the fish), 97% falling between 6 and 9 d; extremes were 3 and 10 d. Otolith ages are expected to underestimate absolute age (from fertilization) by approximately 4 d. Individual larvae collected together exhibited up to a two-fold variation in length, but the otolith ages were often the same. Otolith size, daily growth unit spacing, and subdaily increment structure were markedly different for "fast-" and "slow-growing" larvae.

Forty-four juvenile bluefin tuna (267-413 mm fork length, FL) were also collected by hook and line near Miami (1979, 1980) and examined for otolith growth increments. Total counts on 10 specimens were used to back-calculate a mean birth date (fertilization) of 2 May and a range extending from mid-April to early July. Size and microstructural characteristics of the area encompassing the first 10 increments in otoliths of juveniles were most similar to "fast-growing" larvae in increment spacing and subdaily composition. The evidence suggests that differential survival of larvae is associated with growth rate in the first 2 wk of life.

INTRODUCTION

Several studies have attempted to document the time of spawning and early age and growth of bluefin tuna, *Thunnus thynnus*, in the western Atlantic and Gulf of Mexico. All of these studies have used what may be termed "indirect" approaches, i.e., individual specimens were not aged, but rather inferences were drawn from a time series of collections, often representing relatively few fish. The time of spawning has been estimated from the seasonal occurrence of age 0 bluefin tuna (Rivas 1954; Mather and Schuck 1960; Furnestin and Dardignac 1962; Potthoff and Richards 1970) and from examination of gonadal condition in adults (Rivas 1954; Baglin 1982). Such studies have indicated that spawning occurs in the spring, primarily in April, May, and June. Growth rates during the summer and fall of the first year have been estimated from serial compilations of juvenile length data collected by a variety of techniques, including sampling fish regurgitated by terns (Potthoff and Richards 1970). Laboratory or enclosure rearing of bluefin tuna larvae and juveniles (Sanzo 1932; Harada, Kumai, Mizuno, Murata, Nakamura, Miyashita, and Hurutani 1971) has also contributed to our knowledge of the early life history of this important species. Estimated growth rates are relatively high, as generally expected for scombrid fishes; however, there is considerable variation between studies and no clear-cut data on the shape of the growth curve for the first 2 or 3 mo of life.

Larval fish can be directly aged by examination of their otolith microstructure (Brothers et al. 1976; Methot and Kramer 1979; Kendall and Gordon 1981; Townsend and Graham 1981). With appropriate correction factors, absolute ages and spawning dates can be calculated from otolith daily growth unit counts. Our objective in this study was to apply these techniques to a series of Atlantic bluefin tuna larvae and juveniles for computation of early growth rate and estimation of young-of-year age and spawning time.

METHODS AND MATERIALS

Larvae were collected on four occasions (Table 1), using either surface 1 m conical plankton net tows or a 1 x 2 m neuston sampler, both with 0.947 mm mesh size. Entire samples were immediately preserved in 95% ethanol. A total of 369 fish larvae were later identified as bluefin tuna by T. C. Potthoff and W. J. Richards, NOAA, National Marine Fisheries Service, Southeast Fisheries Center, Miami, Fla. The 95% ethanol-stored larvae were soaked in water for several minutes before measurement and otolith extraction. This procedure reduced some of the shrinkage caused by the alcohol and tended to straighten and soften the bodies. Although shrinkage is known to occur upon preservation of fish larvae (e.g., up to 15% after net handling and Formalin[3] fixation in chub mackerel larvae; Theilacker and Dorsey 1980), we made no corrections in this study. Shrinkage was assumed to be a constant proportion for all fish. These fish were measured (from the tip of the upper jaw to the tip of the notochord, NL; or to developing hypural plates, SL) to the nearest 0.1 mm with an ocular micrometer in a dissecting scope. Otoliths (sagittae and lapilli) were dissected out of the larvae with fine needles (Brothers and McFarland 1981) and then placed in a drop of immersion oil on a microscope slide. Otoliths were examined without any further preparation with a compound light microscope adapted for video viewing. Increment counts and measurements were made off the video monitor at magnifications of either 1,536× or

[1]Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, N.Y.; present address: 3 Sunset West, Ithaca NY 14850.

[2]Southeast Fisheries Center Miami Laboratory, National Marine Fisheries Service, NOAA, 75 Virginia Beach Drive, Miami, FL 33149-1099.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1.—Larval samples of bluefin tuna taken off Miami, Fla., May and June 1981.

Date	Gear	Locality[1]	No. identified	No. for otolith data	Mean length[2] (mm)	Mean otolith age[3] (d)
19 May 1981	1 m conical plankton net; 0.505 mm mesh size	Pacific Light	52	51	5.22	7.90
20 May 1981	1 m conical plankton net; 0.505 mm mesh size	Fowey Light	176	141	5.14	7.80
21 May 1981	1 m conical plankton net; 0.505 mm mesh size	Fowey Light	93	90	4.96	7.26
2 June 1981	1 x 2 m neuston net; 0.947 mm mesh size	Fowey Light	48	46	5.48	6.83

[1]All sampling locations were at the edge of the Gulf Stream (5-10 mi offshore).
[2]Based only on fish used for otolith data.
[3]Not corrected to fertilization.

2,790 × . Otolith radii were measured as the maximum distance from the primordium (central, optically dense area) to the otolith margin, which was usually to the rostrum (especially in larger larvae). In the final analysis of the 369 larvae, only 328 were used because of damage to 41 specimens and resulting uncertainties with regard to fish length.

Hook-and-line caught juvenile bluefin tuna were obtained from charter boats fishing off Miami during the summer and fall of 1979 and 1980. A total of 41 fish (267-413 mm FL) were measured and sampled for sagittae. Otoliths were placed in immersion oil on microscope slides for preliminary viewing. Further preparation was necessary to view otoliths with high magnification since focusing on critical regions was prevented by otolith thickness and projecting surfaces. Two areas of interest were identified for total counts and examination of early larval growth history. Relatively unambiguous and continuous increment counts could be made from the core ("nucleus") to the margin of the antirostrum (terminology of Messieh 1972; also see Glossary). This count was facilitated by breaking the dorsal-posterior quadrant away from the rest of the otolith by slight pressure with a scalpel blade. There was considerable individual variation in the "readability" of the otoliths, which was correlated in part with greater breadth (lateral view) of the antirostrum. As a result of eliminating difficult-to-analyze specimens or those inadequately prepared, only 10 fish were used for total increment counts.

Maximum radius measurements were made from the primordium to the middle of the eighth daily growth unit (defined below). This measurement was made on a total of 25 fish. Breaking away (with scalpel) or grinding down (600 grit Carborundum) of the dorsal-posterior quadrant of the otolith was necessary to focus on the early growth stages.

All statistical inferences were based on a significance level of $\alpha = 0.05$. Line fits were calculated by the method of least squares (Dixon and Massey 1969) or Bartlett's best fit (Bartlett 1949).

RESULTS

Growth increments in the sagittae and lapilli of fish at the middle and upper size ranges represented in our sample are shown in Figure 1. References to otoliths in the remainder of the text refer to the sagitta unless otherwise stated, since most counts and measurements were made on these otoliths. Sagittae are nearly circular or anteriorly elongate ovals when viewed from the interior or exterior (≅ sagittal section) side, flattened, elongated hemispheres when viewed from above or below (≅ frontal section), or flattened hemispheres as seen from an anterior or posterior point of view (≅ transverse section, see Glossary). All microscopic observations were made with larval otoliths lying on the external (distal) face, i.e., flattened or internal (medial) face upwards. Under these viewing conditions, there is an irregularly round, optically dense region in the center of the otolith. This region, approximately 5 μm in diameter, is termed the primordium. Surrounding the primordium are usually two (sometimes three) diffuse and difficult to discern optically dense layers. The first optically dense layer has an average diameter of 12 μm; the second has an average diameter of 18 μm. They are not clearly visible on all specimens. This area, described above, is surrounded by well-defined growth increments. Growth increments are defined as bipartite structures composed of one optically transparent and one less transparent layer. The area circumscribed by the first clear growth increment subunit may be termed the core. Thus, the core includes the primordium and an area of "nonincremental" growth or at least atypical incremental growth. In some specimens, the whole core is more optically dense than surrounding material, although a primordium is still clearly visible (Brothers and McFarland 1981; Tanaka et al. 1981). The incremental subunits are of approximately equal thickness for the first two or three increments, thereafter gradually changing, so that the optically transparent subunit becomes progressively wider relative to the denser subunit. Care has to be taken in observing dark and light subunits since they can reverse appearance under different focusing conditions. Consistent results are obtained at a "high" focal point, i.e., the greatest lens to object distance giving a sharp image. Increments on larval bluefin tuna otoliths appear visibly distinct in nature for most specimens and are structurally analogous to the simple daily growth units seen in many species (Brothers 1979; Pannella 1980). In the largest and apparently fastest growing individuals, this basic pattern is modified in the last three or four increments. Fine increments, termed subdaily (see Fig. 1), appear superimposed over the presumed daily growth unit structures. Subdaily increments are structurally homologous to simple daily growth units. Their presence has been noted in acetate replicas and SEM preparations of this species and other tunas (Brothers[4]). Thus, certain

[4]Brothers, E. B. 1981. Daily growth increments in otoliths from laboratory-reared chub mackerel (*Scomber japonicus*) larvae. Unpubl. manuscr. Cornell University, Corson Hall, Ithaca, NY 14853.

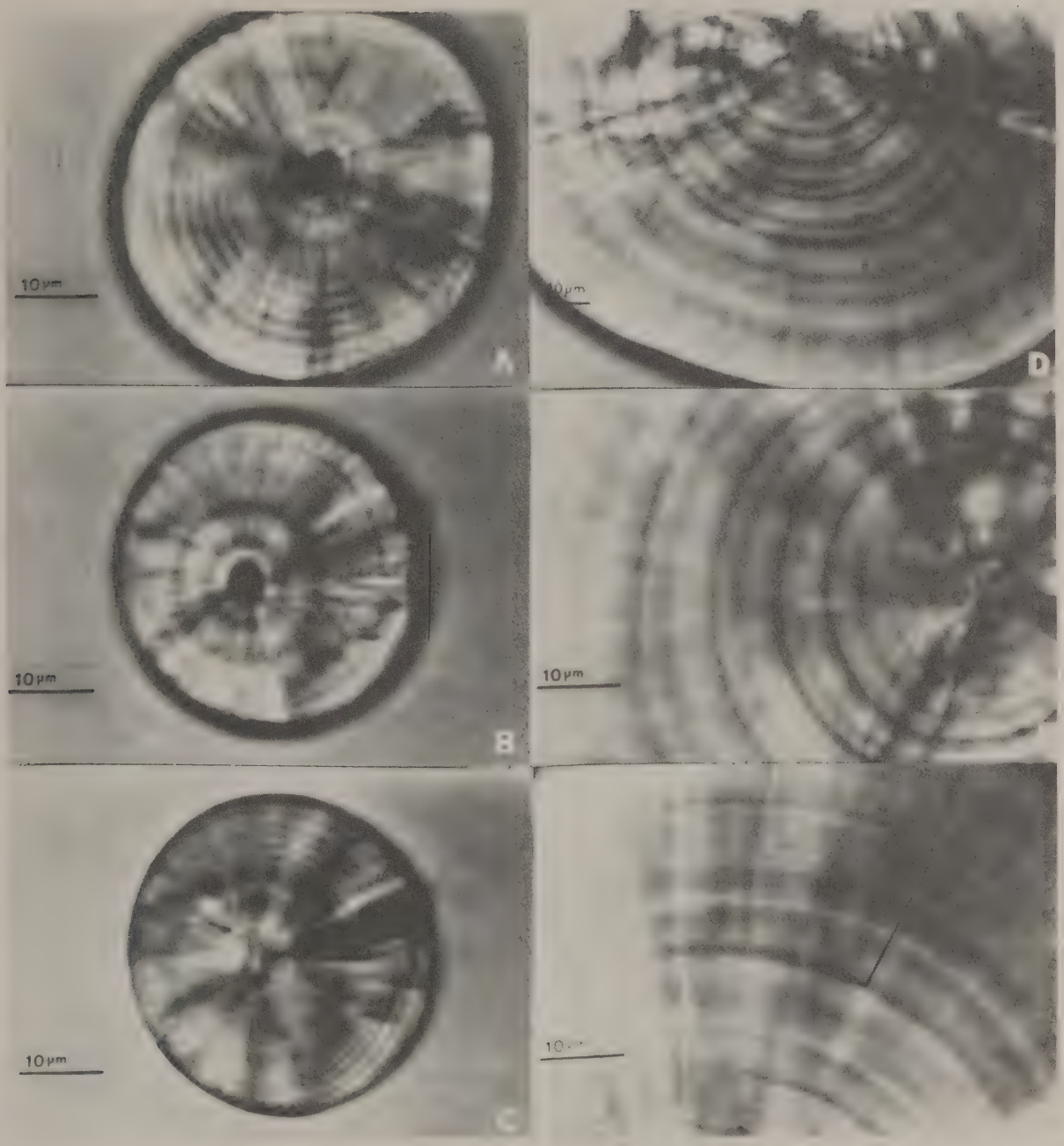

Figure 1.—Microstructure of larval bluefin tuna otoliths. All micrographs were taken from the video monitor: A) Sagitta from 5.3 mm NL larva. Note the dark, central primordium with two diffuse increments surrounding it. There are eight daily growth units around the core. B) Lappilus, same specimen as above. The photo was taken at "high" focus (see text). C) Same as B, but with "low" focus. Note the reversal of light and dark areas in the core and surrounding increments. D) Sagitta from 8.2 mm SL larva. Note lower magnification in comparing with A. E) Higher magnification of above specimen. F) Sagitta of 9 mm larva. Subdaily increments are clearly visible. Bracket indicates one daily growth unit.

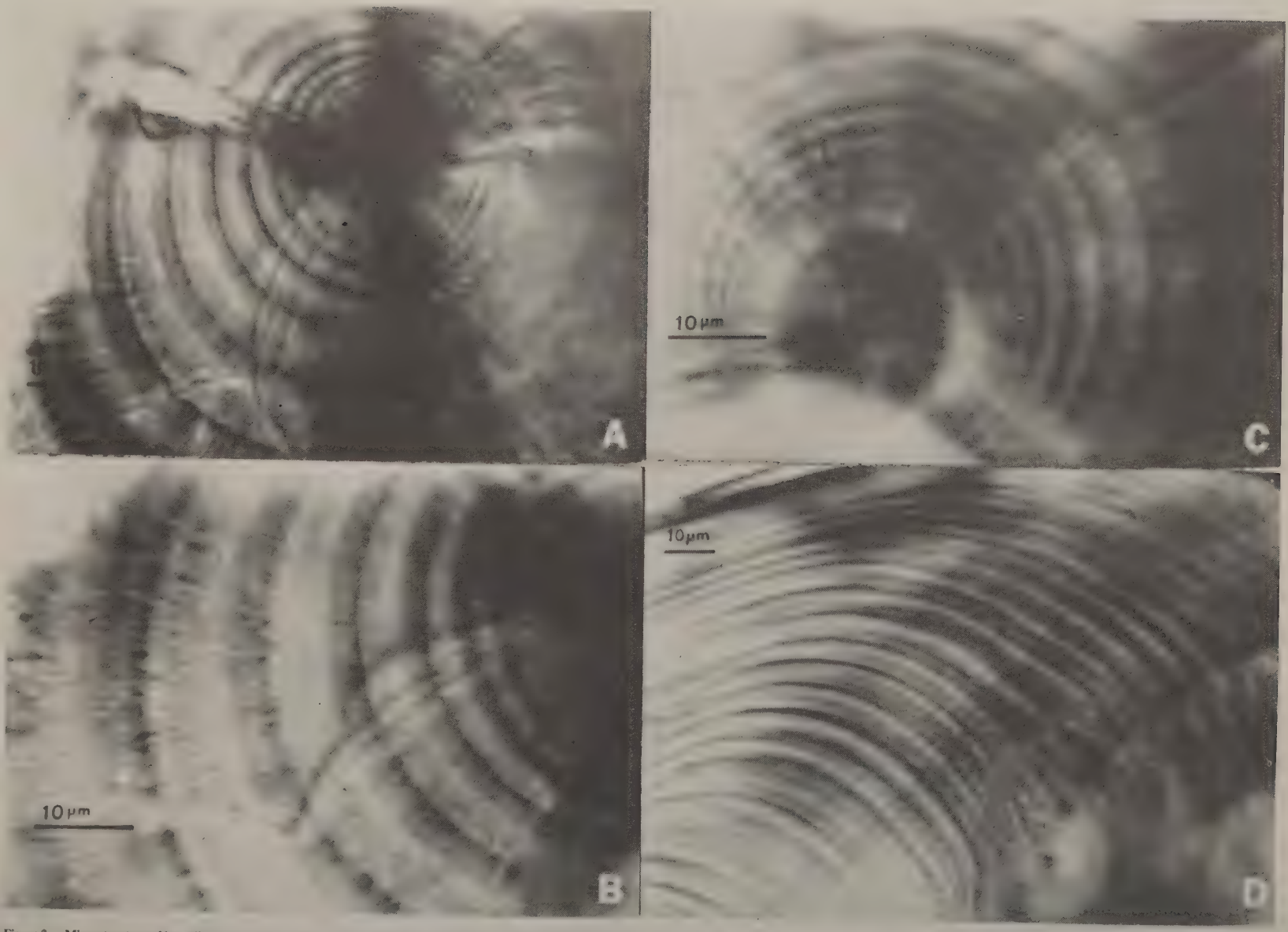

Figure 2.—Microstructure of juvenile bluefin tuna otoliths: A) Central area of 413 mm FL specimen. Compare with D in Figure 1. B) Higher magnification view of same specimen. C) Central area of 344 mm FL juvenile otolith. D) Tip of otolith antirostrum, 306 mm FL juvenile.

daily growth units may be considered complex, comprised of 10 or more fine growth increments.

Since age validation (establishing the time sequence of increment formation, see Glossary) was not accomplished in this study, reference to daily growth increment or unit in the remainder of this paper is a presumption that they are formed on a daily basis unless qualified with the term "subdaily." This is based on the evidence that bluefin tuna microstructural patterns described as growth increments, growth units, or daily growth units are structural homologues of features demonstrated to be formed with diel periodicity in a wide variety of species (Brothers et al. 1976; Taubert and Coble 1977; Barkman 1978; Brothers 1979; Pannella 1980; Brothers and McFarland 1981; and many others), particularly in other scombrids (Wild and Foreman 1980; Uchiyama and Struhsaker 1981). Some of the strongest direct evidence of daily formation of increments in scombrid larvae otoliths was found for laboratory-reared chub mackerel (*Scomber japonicus*; Brothers footnote 4) larvae. Accordingly, we feel that the presumed daily growth units we describe for young-of-the-year bluefin tuna can be used for ageing. The validity of this assumption is addressed in the Discussion section.

The portion of juvenile otoliths corresponding to larval growth (Fig. 2) appeared essentially the same as otoliths from larval fish, with the exception of two features: 1) The growth unit spacing averaged greater for the juveniles, and 2) subdaily increments were more common. Growth units along a counting path from the core to the tip of the antirostrum were highly variable in clarity and thickness. A zone of very thick, optically dense and diffuse growth units begins after approximately the 15th growth unit and continues for a substantial distance, covering 30 or more growth units. Beyond this point, growth units are more distinct and gradually thin as they approach the otolith margin (see Fig. 2).

Growth increment counts on larval otoliths include only presumed daily growth units outside of the core; subdailies are not tallied (Fig. 3). Half of the 328 larval specimens had eight increments and 97% had between six and nine increments. The lowest number recorded was three for a specimen 4.2 mm NL and the highest number was 10 for two specimens 5.6 and 6.5 mm SL. Six specimens over 7 mm SL had counts of either eight or nine. Lapilli from larvae were also examined and were found to have essentially the same primordium and core features. Daily growth unit counts were in good agreement with the sagittae counts, and increments on lapilli were sometimes less ambiguous, especially in larger specimens.

Maximum otolith radius was measured and compared with fish length (Fig. 4). Although there is a clear positive relationship between these two measurements, the form of the best fit line is uncertain. A least squares regression in an exponential form, $y = ae^{bx}$, gave the best fit ($a = 7.02$, $b = 0.24$, and $r^2 = 0.71$) to all the data; however, it appears that the largest and fastest growing fish have reached a transition to another, more rapid otolith growth stanza. The rostral region begins to grow very rapidly in some fish over 7 mm SL. Since maximum diameter was measured, this leads to two of the larvae having what appear to be disproportionately large otoliths. A more representative description of otolith growth would probably be achieved by fitting power functions to individual growth stanzas, but more larvae in the larger size classes are necessary. A final observation on the data in Figure 4 indicates that there may be some slight variation in the relative size of the otoliths as a function of collection date. There are several possible causes for this, including differential fish shrinkage as a function of net handling or time of preservation, or differential otolith growth as a function of fish growth. The variation observed is not great enough to affect our major conclusions on fish growth rates. When radius data for a representative

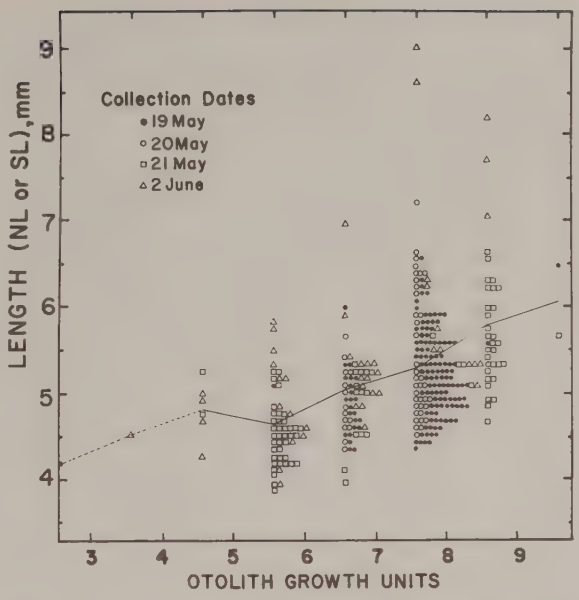

Figure 3.—Growth of Larval bluefin tuna based on otolith growth units. The data are plotted by collection date and are uncorrected for the age of first increment formation. The line connects mean lengths for each "age" group.

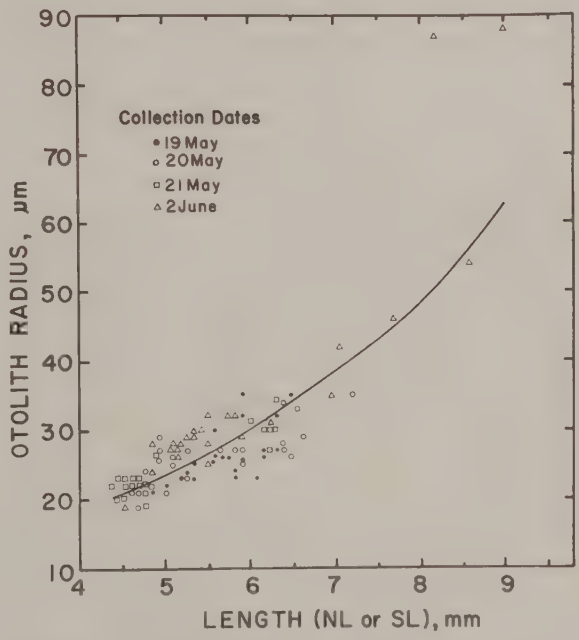

Figure 4.—Relationship between otolith radius and larval length. The line is the least squares regression in the exponential form $y = ae^{bx}$ ($N = 90$, $a = 7.02$, $b = 0.24$, $r^2 = 0.71$).

sample (N = 25) of larval otoliths with eight increments are compared with measurements of the eighth increment on juvenile otoliths, some overlap in the distributions and three exceptional larval points are evident (Fig. 5). In general, juvenile measurements show a larger average otolith size than larvae at the same otolith age.

Otolith ages were adjusted to absolute age from fertilization by a correction factor of 4 d (see Discussion below) and then used to calculate spawning dates for the 1981 samples (Fig. 6). Similar calculations for ten 1979 and 1980 juveniles gave a range of spawning dates from 13 April to 20 May, with a mean date of 2 May.

Juvenile fork lengths were plotted against collection dates (Fig. 7) to yield an estimate of growth rate. For purposes of this analysis, all fish were assumed to have been spawned on the same day of the year, 10 May. A least squares regression line fitted to the data had a slope of 1.15 (r^2 = 0.74). Thus, a linear fit indicated a growth rate of 1.5 mm/d. Adjusted age data for 10 juveniles were also regressed against length to give a slope of 1.39 (r^2 = 0.88). This slope was not significantly different (α = 0.05) from that of the date-length regression.

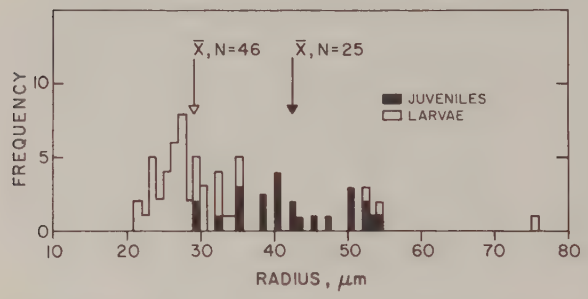

Figure 5.—Frequency distribution of otolith radii for larvae with eight daily growth units (open bars), and radius to the eighth growth unit in otoliths of juveniles (solid bars). The mean radius value and sample size are indicated for both groups.

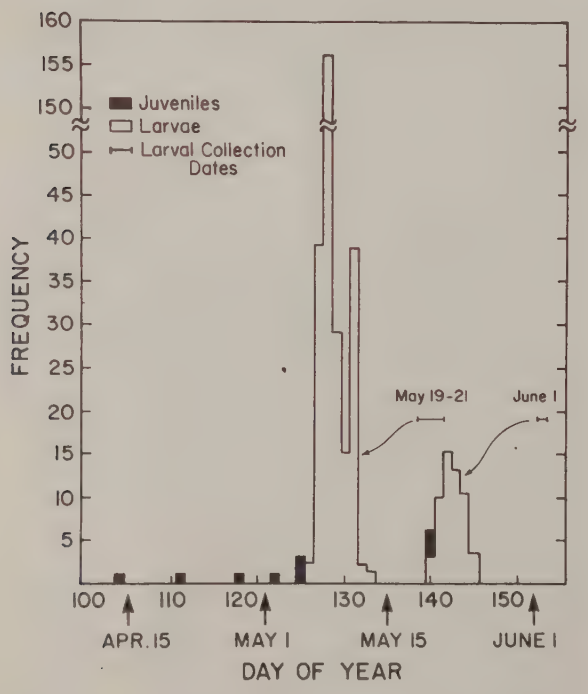

Figure 6.—Back-calculated hatching dates for larvae (solid bars) and juveniles (open bars). Cross bars indicate the dates of larval collections. Otolith ages (daily growth unit counts) were corrected by adding 4 d before determining hatching times.

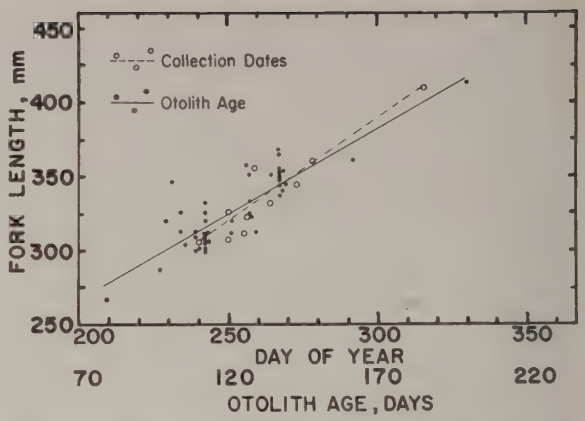

Figure 7.—Growth rate of juvenile bluefin tuna. The solid points are fork lengths plotted against collection date. The line is the calculated least-squares regression (N = 44, y = 37.28 + 1.15x, r^2 = 0.74). The open points and dashed line [by Bartlett's (1949) best fit] are fork length plotted against otolith age (N = 10, y = 151.88 + 1.39x, r^2 = 0.88). The relationship between the two sets of abscissa values assumes an average birth date of 10 May.

DISCUSSION

Implicit in the Results section was our discrimination between growth increments and daily growth units. Both are structurally equivalent with the exception that some growth units were observed to be comprised of finer lamellar structures, provisionally termed subdaily growth increments. In order to validate the temporal nature of increment formation, a number of tests or criteria should be applied (Brothers 1979, 1983). Assuming that all observable growth increments are daily can be highly misleading if in fact subdaily increments are prevalent, as they appear to be in a number of species and growth stages (Brothers footnote 4). Direct validation is often not possible within the scope of a study. Therefore, indirect or comparative methods must be employed to determine the likelihood of whether daily growth units are present and correctly discriminated. The protocol used here is simply for an experienced otolith reader to make counts. Subjective decisions are used when suspected subdaily growth increments are present. A hypothesis is proposed that the counted increments or units are all daily and, therefore, counts can be used to determine age and growth rate. If such counts are accurate representations of age and growth, then they should yield results compatible with other independent measures of age and growth. This is basically the procedure followed here. The otolith reader (Brothers) was experienced with otolith microstructure of many species, including several scombrids, in which daily growth units have been experimentally validated in lab-reared and wild fish. The reader made a decision of what to count based on structural similarity to known daily growth units in other species. Several lines of evidence were then used to compare the otolith microstructure

results with available data on the early life history of bluefin tuna. This is not a totally satisfactory approach since it does contain an element of circularity; however, we feel that the use of independent estimates of age and growth from other studies makes our analysis valid and we are confident that daily growth units have been correctly identified and counted.

Otolith ages were corrected by adding 4 d to total counts. This correction implies that the first counted increment was formed 4 d after spawning or fertilization. Daily growth unit formation can be initiated at a variety of points in ontogeny, based on the species and developmental pattern (Brothers et al. 1976; Barkman 1978; Brothers footnote 4). Otoliths (the sagittae and probably lapilli) are known to be present at hatching in many scombrids (see illustration and description of *Scomber japonicus* in Fritzsche 1978; Radtke 1983; Brothers footnote 4) and specifically in bluefin tuna (Sanzo 1932). Tropical tunas have typically small eggs, short development times, and rapid yolk absorption. For example, in Fritzsche (1978), hatching times for seven tunas averaged just over 1.5 d. Mediterranean bluefin tuna eggs reared by Sanzo (1932) hatched in just over 2 d. Yellowfin tuna, *Thunnus albacares*, eggs hatched in 1 to 1.5 d at 25 °C (Harada, Mizuno, Murata, Miyashita, and Hurutani 1971). The time of yolk absorption and the onset of exogenous feeding are also temperature dependent, but usually commence about 2 d after hatching, as for example in yellowfin tuna (Harada, Mizuno, Murata, Miyashita, and Hurutani 1971). Sanzo (1932) illustrated that a bluefin tuna larva with a well-developed mouth could resorb its yolk 2 to 3 d posthatching. Even in relatively cooler temperatures (19 °C), chub mackerel take only about 4 d from fertilization to reach the feeding stage. At 16 °C, chub mackerel take 3.6 d to hatch and 4 more days to the onset of feeding. Otoliths of chub mackerel larvae reared under these conditions did not form increments until 3 or 4 d from hatching (Brothers footnote 4). Thus, in this species, and in the northern anchovy, *Engraulis mordax*, (Brothers et al. 1976), daily growth units begin to form about the time of the onset of exogenous feeding. Using these data as a guide, adjusting counts upwards by adding four to the otolith counts for bluefin tuna should be very close to the correct value for this species. Radtke (1983) found that in skipjack tuna, *Euthynnus pelamis*, the first increment formed 1 d after hatching, or approximately 3 d from fertilization. The two or three diffuse, poorly defined intracore "increments" likely correspond to preyolk absorption and possible prehatching daily marks. Similar features are very common in a wide variety of other species, and some have been demonstrated to be daily in formation (Brothers footnote 4).

Back calculation from total corrected ages to fertilization dates for larvae results in two peaks centering on 8 and 22 May (Fig. 6). Back calculations from juvenile ages (N = 10) give fertilization dates overlapping with the larval data. This result is in complete agreement with several other studies that have examined adult gonads or the seasonal appearance of larvae (Rivas 1954; Mather and Schuck 1960; Tiews 1963; Potthoff and Richards 1970; Richards 1976; Mather[3]; and others summarized in Fritzsche 1978). Thus, the hypothesis that the counted structures are in fact daily growth units is supported by the spawning date analysis.

A second line of evidence for the daily nature of the counted growth units is based on two calculations of growth rates for juveniles. The first assumes that fish were spawned at approximately the same time and that a temporal series of specimens represents fish of successive ages. Illustrated in Figure 7 is the calculated slope for the juvenile series used in this paper, compared with a linear estimate of growth rate derived from otolith counts. The slopes are not significantly different and, therefore, these independent measures of growth rate are in agreement. If a linear growth rate is accepted as a reasonable approximation for the pattern, at least during the late summer and early fall, then we can compare our estimate for juveniles of between 1.0 and 1.5 mm/d with those of previous studies on bluefin tuna (Table 2). Even for only 10 fish, growth unit counts give results (1.4 mm/d) that are clearly compatible with other studies (Table 2) and are in agreement with the hypothesis of daily increment formation.

How do these data for bluefin tuna young-of-the-year growth rates compare with published studies on bluefin tuna and other scombrids? Although there is general agreement on juvenile ($>\sim$ 30 cm FL) rates, there is much more uncertainty on the shape of the curve in the first 3 mo of life. Starting with the well-supported evidence that fish about 100 d old (mid-August) are approximately 300 mm FL, then larvae and postlarvae must have an average growth rate of 3 mm/d. But is growth during this period following a linear or curvilinear form? Illustrated in Figure 8 are examples of early growth data for bluefin and other related species. The various studies are based on a

[3]Mather, F. J., III, Associate Scientist, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, pers. commun. 1982.

Table 2.—Average growth rates for juvenile bluefin tuna, mid-August to mid-October.

Source	Method	Length range FL (mm)	Rate (mm/d)
This study	Size progression	267-413	[1]1.15 ± .21
	Otoliths	306-413	[1]1.39 ± .40
Mather and Schuck (1960)	Size progression	45-450	1.1
Rivas (1954)	Size progression	20-460	1.0
Rivas[2]	Size progression	15-450	1.4
Furnestin and Dardignac (1962)	Size progression (eastern Atlantic)	310-450	1.6
Harada, Kumai, Mizuno, Murata, Nakamura, Miyashita, and Hurutani (1971)	Rearing in enclosures (Pacific)	250-410	1.8

[1]Mean ± 95% confidence limits.

[2]Rivas, L. R., Nova University, Ocean Science Center, 8000 North Ocean Dr., Dania, FL 33004, pers. commun. 1982.

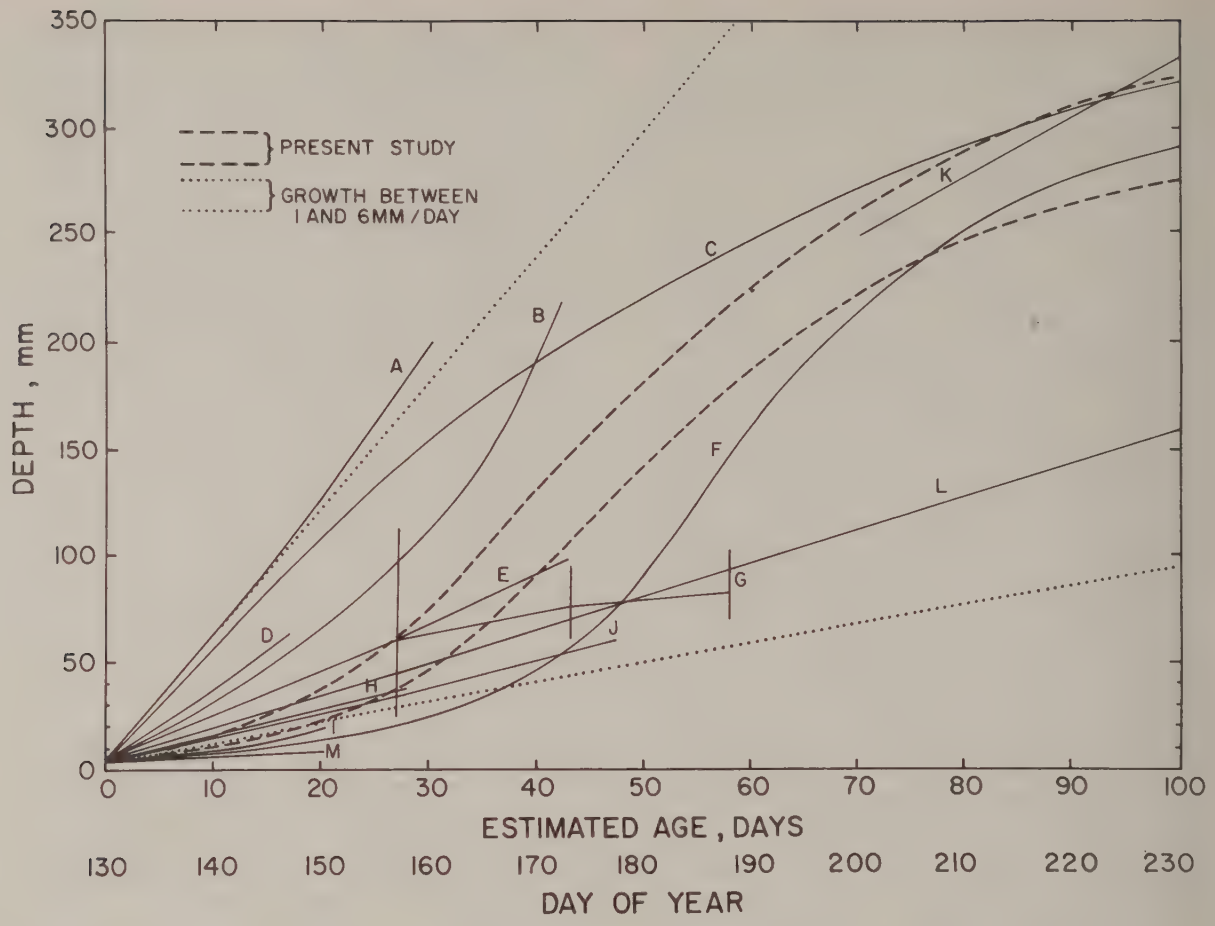

Figure 8.—Sampling of early growth rate data for bluefin tuna and other scombrid fishes. Length measures varied slightly in different studies and with different sized fish (i.e., NL, SL, and FL). Several lines are only approximations from endpoints to show average rate (A, D, E, H. L). All fish were arbitrarily assigned a birth date of 10 May to facilitate comparisons with bluefin tuna (A, C, F, G, K, and dashed lines). Upper and lower estimates of size for age for bluefin tuna in the present study are indicated by the dashed lines. These values assume a single hatching date, thus considerably more variation would result if spawning continued over a month or more. The dotted lines define growth rates between 1 and 6 mm/d. The species and sources are as follows: A) Bluefin tuna, field, Rivas (1954); B) *Sarda orientalis*, lab, Harada et al. (1974); C) bluefin tuna, field, Mather and Schuck (1960); D) *Auxis thazard*, lab, Harada, Murata, and Miyashita (1973); E) *Auxis tapeinosoma*, lab, Harada, Murata, and Furutani (1973); F) bluefin tuna, field, Rivas (pers. commun. 1982); G) bluefin tuna, field (tern stomachs), Potthoff and Richards (1970); H) *Scomberomorus cavalla*, field, Dwinnell and Futch (1973); I) *Scomber japonicus*, lab, Hunter and Kimbrell (1980); J) *Scomber scombrus*, field (otoliths), Kendall and Gordon (1981); K) bluefin tuna (Pacific), enclosure, Harada, Kumai, Mizuno, Murata, Nakamura, Miyashita, and Hurutani (1971); L) *Katsuwonus pelamis*, field (otoliths), Uchiyama and Struhsaker (1981); M) *Thunnus albacares*, lab, Harada, Mizuno, Murata, Miyashita, and Hurutani (1971).

number of techniques including laboratory and enclosure rearing, length progression, and otolith analysis. Growth rates after the first month may be < 1 mm/d to over 6 mm/d. When scombrid larval growth has been examined in detail in the laboratory, as for the chub mackerel (Hunter and Kimbrell 1980), a relatively slow-growth phase (~ 0.25 mm/d) for the first 8 to 10 d is followed by a rapid acceleration to a rate of 0.9 to 3.2 mm/d (dependent on temperature). The high-growth rates then show an exponential decay to the lower juvenile rates. This general form of growth curve may be common for fish larvae in general (Zweifel and Lasker 1976), although the parameters are expected to vary widely. Using the otolith data available for this study, a curve of this general form was extrapolated from our largest larvae through the lower end of our juvenile data (dashed lines on Fig. 8). The growth rates derived from such a curve are well within the limits suggested by other studies. The position of some of these curves is the result of other authors also extrapolating over size ranges where data were absent, but assuming a different shape to the curve (Mather and Schuck 1960). Other problems are likely to arise due to selective sampling of smaller individuals (both younger and slower growing). For example, in the data of Potthoff and Richards (1970), a very low growth rate is indicated (0.73 mm/d), which probably arose from differential availability of small versus large juveniles to the terns they sampled. Their June sample appears to be fairly representative of the expected size range; however, the latter two samples are probably poorly underestimating larger individuals. There is reason to believe that differences in catchability of larger larvae also affect our data. Only the higher speed neuston sample contained individuals (5) over 7.5 mm SL, even though some of the other plankton net collections contained more than three times as many

larvae. The otolith data are clearly on the "right track"; however, further refinements of the growth curve for the first 3 mo will require additional collections of fish in the size range of 20 to 250 mm FL.

An interesting feature of the larval otolith data is the great variation in length for a given age. At an adjusted otolith age of 12 d, larvae were from 4.5 to 9.0 mm SL, differing by a factor of two. If body mass was considered, the range is likely to be from an order of a magnitude to 15 ×, since changes in body shape during these sizes result in length-mass relationships with exponents of approximately four (even for chub mackerel, which does not undergo as dramatic a change in head and body form; Hunter and Kimbrell 1980). Thus, there appears to be a large spectrum of growth rates with some individuals on the "fast" end and others on the "slow" end. Ototlith size and daily growth unit spacing are broadly related to length of larval bluefin tuna (Fig. 4). Therefore, examination of early otolith characteristics of juveniles provides an opportunity to determine whether both "fast-" and "slow-growing" larvae are represented in those fishes that have survived the larval and postlarval stages. Although sample sizes for the juveniles are small and restricted in geographic coverage, preliminary indications are that juveniles had a larval growth history more indicative of "fast" growers than the more commonly collected "slow" growers. Because of likely size sampling biases for the larvae, conclusive statements on the relative abundance of fast- and slow-growing larvae are difficult; however, the data support the intuitive hypothesis that rapid early larval growth is related to a greater probability of survival to the juvenile stage. Two basic mechanisms may operate independently or in combination to effect higher mortality rates as an inverse function of growth rate: 1) Starvation and 2) predation (including cannibalism). Undernourished fish larvae, not encountering adequate concentrations or size distributions of food organisms, may reach a "point of no return" (Blaxter and Hempel 1963), and suffer high mortality as a result of physiological failure and increased susceptibility to disease, parasitism, and predation. The "critical period" of Hjort (1914) and subsequent authors (May 1974; Sharp 1980) is an expression of the extreme sensitivity of the early stages, immediately after yolk absorption. There are a number of well-documented cases of this phenomenon in the laboratory and field (Hunter 1972; Lasker and Zweifel 1978; O'Connell 1980; Theilacker and Dorsey 1980; Lasker 1981), and Hunter and Kimbrell (1980) have noted that starvation was irreversible for chub mackerel larvae if they were not feeding by day 4 or 5 (19 °C). Bluefin tuna larvae are likely to be subjected to predation by a wide variety of other fishes, invertebrates, and particularly conspecific larvae and postlarvae. The smaller a larva is and the slower it grows, the greater the number of potential predators and the longer the period of intense predation. This effect may be particularly severe when one of the important predators are conspecifics in relatively high abundance due to localized spawning. Piscivory and cannibalism were significant features of laboratory-reared chub mackerel feeding behavior (Hunter and Kimbrell 1980) when mean length of fish averaged 8 mm SL. Cannibals as small as 10 mm SL could consume other fish 6 mm NL long. Mouth width was found to be a major determinant of prey size in these fishes. We do not have mouth width data for bluefin tuna, but our measurements of upper jaw length in bluefin tuna larvae should be correlated to gape size. For 5 mm SL bluefin tuna, the upper jaw is approximately 24% of the body length and 28% for 10 mm SL fish. By comparison, chub mackerel have an upper jaw length of 15 to 16% for the same-sized fish. A field study on the food and feeding of Atlantic mackerel, *Scomber scombrus*, by Grave (1981) demonstrated the high incidence of cannibalism in this species. For larvae and juveniles 13-19 mm SL, 83% of their prey were mackerel larvae. Mackerel larvae as small as 10 mm SL were found to consume conspecifics almost half their size. Thus, it is clear that bluefin tuna larvae have a greater potential for piscivory and cannibalism at even smaller sizes than the mackerel. Mayo (1973) noted that *Euthynnus alletteratus*, *Scomberomorus cavalla*, *S. regalis*, and *Auxis* sp. became cannibalistic at about 5 mm SL. The early availability of larger prey and the onset of piscivory (including cannibalism) seem to be necessary conditions for the successful growth and survival of chub mackerel larvae (Hunter and Kimbrell 1980). Such a statement probably holds for bluefin tuna, and all indications are that the requirements are even more restrictive for this species.

One possible interpretation of the data presented in Figure 3, in light of our observations on growth rates of successful recruits, is that many, if not most, of the larvae at the lower ends of the size distributions for each age are doomed—perhaps to fall prey to a variety of predators, including faster growing members of their cohort. Are those fish that appear to be experiencing a very rapid acceleration of growth the ones which have gotten a head start by an earlier onset of piscivory? We do not know the source of the variation in very early growth. Chance exposure to patchy food sources may play a role, as may slight differences in egg and hatching size (Bagenal 1971; Theilacker and Dorsey 1980), but we have no data to support either hypothesis. We cannot take the relative proportion of fast and slow growers in our larval sample as a direct measure of differential mortality, because sampling biases are almost certainly affected by total numbers for each size. The very smallest larvae, 3.0 to 3.8 mm NL, are subject to net extrusion and may be underrepresented. Small- to intermediate-sized (<7.5 mm SL) larvae are probably overrepresented and the average growth rate calculated from the sample would be low. The indicated mean growth rate for the whole sample is subject to other biases which may have the opposite effect of size selection. If size-selective mortality has already occurred when the larvae are 8 or 9 d old, then the sample growth rate may overestimate the average rate for all larvae. These uncertain and conflicting sources of bias have to be considered in interpreting the observed larval growth rates (see Methot and Kramer 1979 for a discussion of similar problems in the northern anchovy).

In summary, analysis of otolith microstructure for larval and juvenile bluefin tuna indicates that daily growth units are present and can be used to age individuals with a high degree of accuracy. Early larvae show a substantial amount of variation in growth rate. Preliminary results suggest that fast growth is correlated with a likelihood of survival to the juvenile stage.

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Interpretation of Growth Bands on Vertebrae and Otoliths of Atlantic Bluefin Tuna, *Thunnus thynnus*

DENNIS W. LEE, ERIC D. PRINCE, and MICHAEL E. CROW[1]

ABSTRACT

Vertebrae and otoliths were collected from 2,287 western Atlantic bluefin tuna, *Thunnus thynnus*, to gain insight into problems associated with interpretating growth bands on giant-size (≥ 209 cm fork length) fish. Examination of terminal growth in otoliths from 554 giant bluefin tuna suggested two major annual slow-growing periods from January through October. Skeletal hardparts were selected from 20 giant female bluefin tuna to determine if more than one growth band forms on otoliths each year after maturity (about 8-10 yr). A total and revised count (2 bands per year) of bands on otoliths and counts of bands from the 35th and 36th vertebrae were used to estimate age, and the results were compared with length-at-age relationships of nine other studies. None of the four counting methods approximated previous work very closely, but the estimates of age based on revised counts of bands on otoliths compared equally well with estimates of age based on the other methods.

Tagging records from a giant bluefin tuna recaptured after almost 16 yr indicated an age of 18+ yr. Counts of growth bands on the 36th vertebra from this fish resulted in an age estimate of 15+ yr. A correction factor for approximating age to the 35th vertebra revised this estimate to 17+ yr, which underestimated age based on tagging records by 1+ yr. Early growth bands (1-5) appeared to be correctly interpreted and errors in the age estimates were probably due to miscounting bands on the outer margin. These data also indicate that growth bands on giant bluefin tuna vertebrae, including the closely spaced bands at the outer margin, should be interpreted as equal to *at least* 1 yr each.

INTRODUCTION

Atlantic bluefin tuna, *Thunnus thynnus*, support important recreational and commercial fisheries that have recently shown signs of decline (Mather 1974; Caddy and Butler 1976; Butler 1982). Since 1970, the International Commission for the Conservation of Atlantic Tunas (ICCAT) has been responsible for recommending policies for management of this species (ICCAT 1971). Biological studies on age and growth are an integral part of stock assessments necessary to formulate management recommendations made by the Commission.

Problems inherent in estimating the age of fish increase as age increases (Pannella 1980) and become severe for long-lived fish, such as bluefin tuna, which may live 30 yr or more (Caddy and Butler 1976; Butler et al. 1977). The age and growth of Atlantic bluefin tuna have been studied since the 1920's, using skeletal hardparts such as vertebrae, otoliths (sagittae), dorsal spines, and scales (Sella 1929; Westman and Gilbert 1941; Mather and Schuck 1960; Rodriguez-Roda 1964, 1971; Nichy and Berry 1976; Butler et al. 1977; Compeán-Jimenez and Bard 1980; Farrugio 1980; Farber and Lee 1981; Hurley et al. 1981). Papers from this volume addressing bluefin tuna include Brothers et al. (1983), Hurley and Iles (1983), and Compeán-Jimenez and Bard (1983). Although many studies show close agreement in ageing young fish (< 6 yr old), authors have noted difficulty in interpreting growth bands on skeletal hardparts of tuna older than ages 6-10.

For example, Mather and Schuck (1960) reported that caudal vertebrae and scales provided reasonable estimates of age for Atlantic bluefin tuna up to 10-12 yr, but these structures were unreliable for older fish. In addition, Caddy and Butler (1976) reported estimates of age for bluefin tuna based on vertebrae and otoliths from the same fish. They observed good agreement up to age 16, but for older fish, age estimates based on interpreting the crowded growth bands at the outer margin of vertebrae were cited as the reason for these discrepancies. Mather and Schuck (1960) and Rodriguez-Roda (1964) also recognized the difficulty of interpreting the crowded growth bands evident on the outer margin of vertebrae after the 8th or 9th band. Caddy and Butler (1976) had greater confidence in estimates from otoliths because presumed year marks were approximately constant in width after age 9. However, the consistent spacing of growth bands on giant bluefin tuna otoliths has not resulted in a consensus on the accuracy of subsequent age estimates. Berry et al. (1977) speculated that there was a tendency when using otoliths to overestimate the age of giant bluefin tuna by as much as 10 yr, because after about age 10, annual marks consist of a "pair of paired" bands (e.g., two or more pairs of translucent and opaque bands for each year of life). Compeán-Jimenez and Bard (1980, 1983) also reported that more than one growth band a year was found on dorsal spines of Atlantic bluefin tuna from the eastern Atlantic Ocean and Mediterranean Sea. Accordingly, the relationship between the rhythmic marks found on vertebrae and otoliths of giant bluefin tuna and specific time sequences generally have not been established. These problems are summarized from the literature as follows:

1) ***Vertebrae tend to underestimate age.*** Growth bands on vertebrae are well-defined up to estimated ages 9-10, but beyond this, results are unclear due to the crowded banding on the centrum margin.
2) ***Otoliths tend to overestimate age.*** Growth bands on otoliths are vague up to about estimated ages 9-10.

[1]Southeast Fisheries Center Miami Laboratory, National Marine Fisheries Service, NOAA, 75 Virginia Beach Drive, Miami, FL 33149-1099.

Although these bands are well-defined and have a consistent width beyond this point, two or more bands may be deposited for each year of life thereafter.

Our objective in this paper is to provide insight into interpreting the growth bands on vertebrae and otoliths of Atlantic bluefin tuna by: 1) Analyzing the growth bands on a vertebra obtained from a tag-recaptured giant bluefin tuna where age is known from tagging records, and 2) assessing the possibility that two or more bands are formed on otoliths from giant bluefin tuna for each year of life after about age 10.

MATERIALS AND METHODS

Vertebrae and otoliths were collected from United States and Japanese commercial catches and U.S. recreational catches of western Atlantic bluefin tuna from the Gulf of Mexico, the Florida Straits, and the western North Atlantic Ocean (North Carolina to Prince Edward Island, Canada) since 1975. No fish were collected in November and December, when bluefin tuna move off the northeast coast and migrate to overwintering grounds (Rivas 1978). The collection of vertebrae, otoliths, and the complete array of supplemental data (fork length, round weight, sex, date captured) were not always obtained for all fish sampled. However, the accumulation of samples from 2,287 bluefin tuna (4.7-280.0 cm fork length, FL) since 1975 has allowed us to examine some of the problems associated with interpreting growth bands on vertebrae and otoliths.

Vertebrae

Caudal peduncles containing the 33rd-36th vertebrae were removed from bluefin tuna following the procedures of Nichy and Berry (1976) and Prince and Lee (1980). The techniques of Berry et al. (1977) for preparing and staining (alizarin red) vertebrae were adopted for this study. The following measurements were taken from each vertebra (in millimeters) with a plastic ruler: 1) Vertebral cone radius—the distance from the focus to the outside rim of the cone, and 2) size of annulus—the distance from the focus to the outside edge of each growth band. The term annulus is used in the remainder of this paper to refer to rhythmic growth increments or bands on vertebrae and otoliths, but the formation of these bands (annuli) may not necessarily coincide with annual events. The morphology of bluefin tuna vertebrae prevents light penetration; therefore, growth bands viewed on the vertebral cone surface consist of one alternating bony ridge and valley. Conversely, growth bands on sectioned otoliths, which allow light penetration (discussed in next section), consist of one alternating translucent and opaque zone (see Glossary).

The relationship between fork length of bluefin tuna and vertebral cone radius was examined for three categories: 1) All sizes (4.7 to 280 cm FL); 2) small- and medium-sizes (4.7 to 208 cm FL); and 3) giant-size fish (209 to 280 cm FL). Statistical inferences for all regression analyses were based on a significance level of $\alpha = 0.05$.

We obtained the caudal peduncle from a giant bluefin tuna tagged (no. 01171) off New Jersey by Canadian biologists on 5 August 1965 and recaptured in the Bahamas on sport gear on 28 May 1981, 15.8 yr later. The 36th vertebra was the only skeletal hardpart we were able to recover. The fish was reported to be 80 cm FL at release and the round weight at recapture was 224 kg (493 lb). A direct measure of length at recapture was not obtained, but we estimated recapture length from a photograph of the fish by using the known length of the forearm and height of a woman standing alongside.

The 35th vertebra has been the primary source of vertebrae age information for Atlantic bluefin tuna in recent years (Berry et al. 1977; Farber and Lee 1981). To relate our findings from the tag-recaptured fish to the literature, we revised the estimated age from the 36th vertebra to approximate that of the 35th using a correction factor (1.7 yr) based on the mean differences between annuli counts of the two vertebrae from 20 giant bluefin tuna (Table 1). Accuracy of our interpretation of growth bands on the 36th vertebra was examined by comparing the measurement of each annulus of this vertebra with mean focus-annuli calculations of the 35th vertebra from 1,029 bluefin tuna having 1 to 17 annuli.

Table 1.—Mean absolute difference, standard deviation of difference, and *t* values from regression analysis (Ho: slope = 1.0) for all pair-wise comparisons of vertebrae and otolith counts of annuli of 20 female giant Atlantic bluefin tuna. Estimates of age were based on the assumption that one annulus is equal to 1 year for counts on the 35th and 36th vertebrae and total otolith. The revised otolith counts were modified by counting two annuli (bands) for each year after the 10th annulus.

Age estimate comparisons	Mean absolute difference (yr)	Standard deviation of difference	*t* values[1]
35th vertebra vs. revised otolith	0.95	0.6	2.74
35th vs. 36th vertebrae	1.65	1.0	2.15
36th vertebra vs. revised otolith	1.70	1.4	3.70
35th vertebra vs. total otolith	6.15	3.5	12.40
Total otolith vs. revised otolith	6.20	2.7	21.90
36th vertebra vs. total otolith	7.80	3.9	12.20

[1]Critical *t* values: *t* value > 2.10 sig. ≤ 0.05, *t* value > 2.88 sig. ≤ 0.01 (Steel and Torrie 1960).

Otoliths

Otoliths (sagittae) were removed from the head of bluefin tuna according to procedures outlined by Nichy and Berry (1976) and Prince and Lee (1980). Otolith length was measured with an ocular micrometer by recording the distance (in micrometer units and converting to millimeters) from the rostrum to the postrostrum (Fig. 1). The relationship between fork length of bluefin tuna and otolith length was examined using regression analyses for three size categories (as previously stated for vertebrae, except smallest size began at 30 cm FL).

Sagittae were prepared and sectioned in a transverse plane (Fig. 1) as described by Berry et al. (1977) and otolith terminologies follow those suggested by Hunt (1978). Sections were made with a variable speed Isomet[2] saw and averaged 0.34 mm thick. At least three sections through the focus of each sagitta (Fig. 1) were mounted on slides. The last growth zone at the terminal edge of medial-ventral (long arm) and medial-dorsal (short arm) ridges of sectioned sagittae from 554 specimens were examined by two independent readers for opaque (as-

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

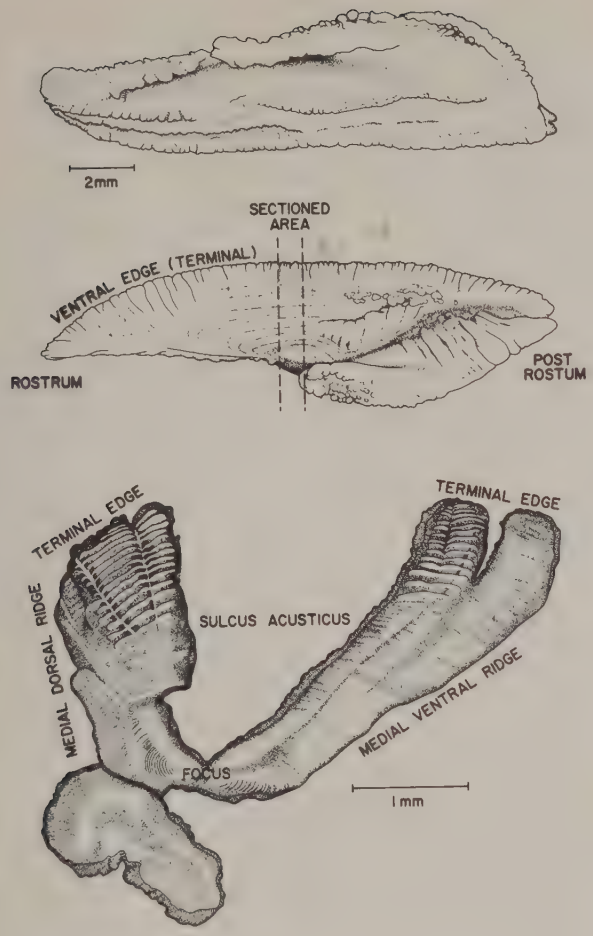

Figure 1.—Proximal and distal view of whole right sagitta otolith (top) and cross section of sagitta otolith (bottom) from a giant Atlantic bluefin tuna.

sumed to represent fast growth) or translucent (assumed to represent slow growth) characteristics. These data gave an indication of the state of growth at time of capture. When viewing sagittae sections with a compound microscope (1,000×) under reflected light with a dark background, opaque zones exhibited a broad white band, while translucent zones appeared as thin dark areas (Blacker 1974; Pannella 1980). If the terminal edge was found to be translucent, the widest point of this zone was measured (1,000×) with an ocular micrometer to the nearest half unit. Mean and variance calculations were computed for monthly samples, and differences in monthly translucent measurements were tested with a Kruskal-Wallis sign rank test (Hollander and Wolfe 1973) to examine seasonal growth.

To investigate whether more than one annual growth band is formed on giant bluefin tuna otoliths, the age of 20 female giant bluefin tuna (from 221 to 278 cm FL) were estimated using a total count of growth bands on the long arm of sectioned sagitta and a revised count, where after the 10th band, each band was equal to 0.5 yr. In addition, the 35th and 36th vertebrae were also analyzed from the 20 fish for comparative purposes and this resulted in four estimates of age for each fish: 1) Total count on sagitta, 2) revised count on sagitta, 3) 35th vertebra count, and 4) 36th vertebra count. The null hypothesis that there was no difference in the counts of annuli from the four methods was tested, using all possible pair-wise regressions of hardpart counts and testing each of the slopes against unity and the intercepts against zero. We used the length-at-age relationships for bluefin tuna developed in nine separate studies (although age validation was not accomplished in any of them) to compare the results of our four methods of ageing with previous work. Mean differences were computed using each of our four ageing methods to determine how close the predicted length-at-age from each of the published relationships compared with the observed length-at-age.

RESULTS

Relationship Between Size of Fish and Size of Hardparts

The linear relationship between fork length and vertebral cone radius was significant for all sizes of bluefin tuna (r^2 = 0.99, Fig. 2A) and the small and medium category (r^2 = 0.98, Fig. 2B). A separate analysis on giant-size fish also demonstrated a significant relationship (r^2 = 0.71, Fig. 2C), but fork length and vertebral cone radius were not as closely related as in other size categories.

A significant relationship was detected between fork length and otolith length for all sizes (r^2 = 0.93, Fig. 3A) and the small and medium category (r^2 = 0.94, Fig. 3B). These relationships appear to fit a curvilinear model better than a linear model (r^2 = 0.97 for both, Fig. 2A, B). When giant-size fish were analyzed separately, a significant relationship was not evident (r^2 = 0.10, Fig 3C).

Vertebrae

Estimated recapture length of the tagged giant bluefin tuna was 254 cm FL, using the known height of the woman (155 cm) in the photograph and 257 cm FL, using the known length of the woman's forearm (22.9 cm). We feel that the forearm estimate was more reliable, since the woman's height in the photograph was cut off at the ankles and could have caused an underestimate of recapture length. Tagging data (Hurley and Iles 1982) supported an age of 18+ yr, based on the reported size-at-tagging (80 cm FL), the presumed hatching months of May or June for western Atlantic bluefin tuna (Richards 1976), the original tagging date of 5 August 1965, and the time-at-large (15 yr 10 mo).

A total count of annuli on the anterior cone surface (including closely spaced bands on the outer margin) of the 36th vertebra resulted in an age estimate of 15+ yr. We found that when the 35th and 36th vertebrae of the 20 tuna were examined, the 36th vertebra underestimated the assigned age of the 35th by an average of about 1.7 yr (Table 1). Therefore, we revised our original age estimate by incorporating this correction factor. The revised estimate (16.7+ or 17 yr) still underestimated the known age based on tagging records by 1+ yr. Measurements from the focus to each annulus on the 36th vertebra of the tagged fish and mean calculations from the 35th vertebra of 1,029 bluefin tuna demonstrated that differences between these measurements become quite large after the 5th annulus (Fig. 4). Dunn's multiple comparison analysis (Hollander and Wolfe 1973) between median focus-annuli measurements on the 35th

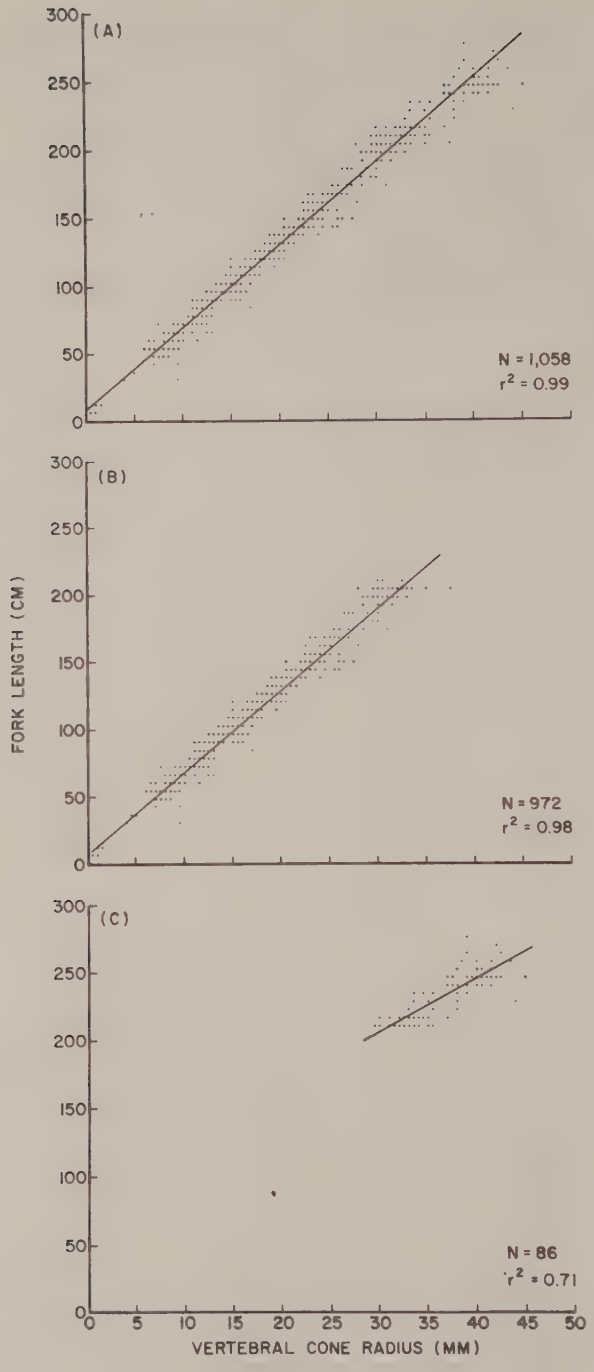

Figure 2.—Relationship of fork length (cm) and vertebral cone radius (mm) for all sizes (A), small- and medium-sizes (B), and giant (C) western Atlantic bluefin tuna.

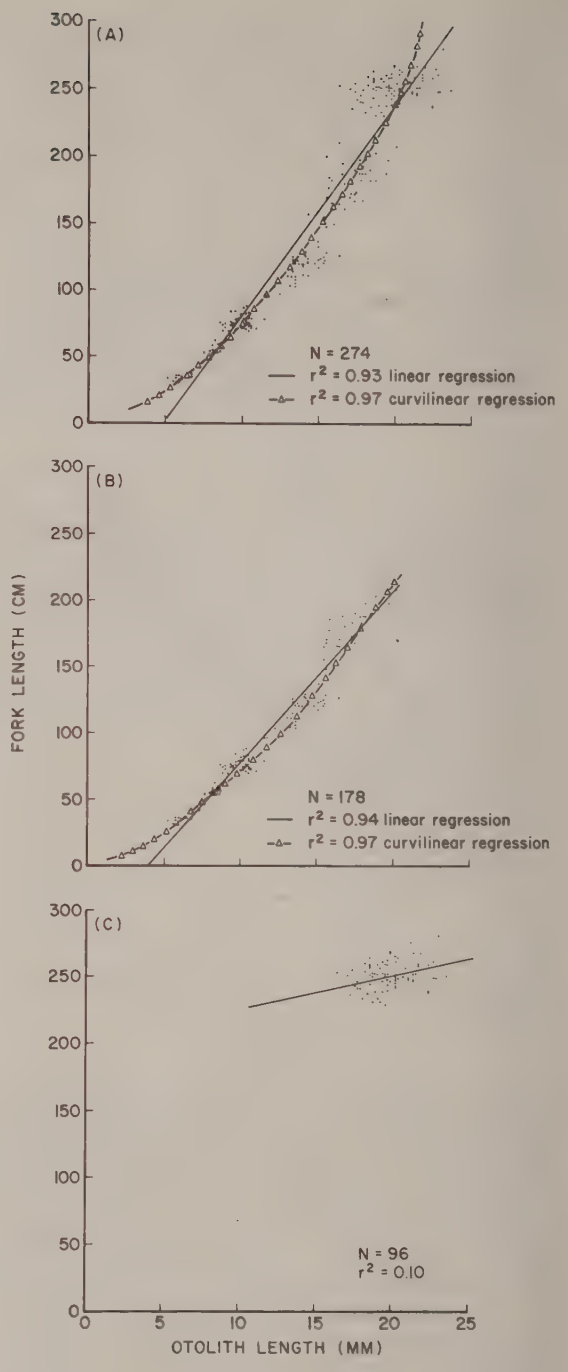

Figure 3.—Relationship of fork length (cm) and otolith length (mm) for all sizes (A), small- and medium-sizes (B), and giant (C) western Atlantic bluefin tuna.

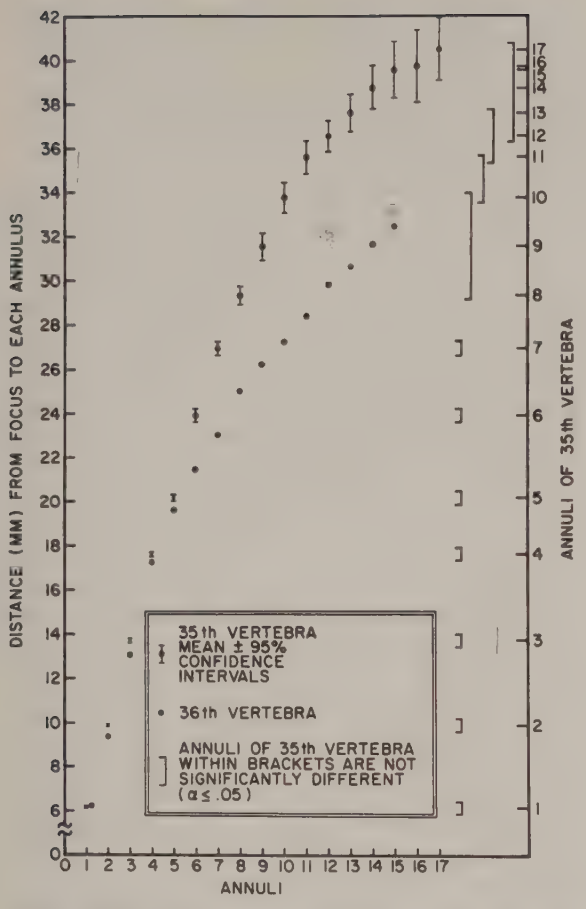
DISTANCE (MM) FROM FOCUS TO EACH ANNULUS
ANNULI OF 35th VERTEBRA
ANNULI
35th VERTEBRA MEAN ± 95% CONFIDENCE INTERVALS
36th VERTEBRA
ANNULI OF 35th VERTEBRA WITHIN BRACKETS ARE NOT SIGNIFICANTLY DIFFERENT (α ≤ .05)

Figure 4.—Distance (mm) from focus to each annulus on the 35th vertebra (mean ± 95% confidence interval) and 36th vertebra (from a single-tagged fish) for western Atlantic bluefin tuna. Multiple comparisons based on Kruskal-Wallis rank sums using Dunn's procedure (Hollander and Wolfe 1973) are shown with mean annuli (35th vertebra) within brackets not significantly different ($\alpha \leq 0.05$).

vertebra indicate that after the 8th annulus, it is increasingly difficult to differentiate annuli on the basis of measurements (Fig. 4). Differentiation between measurements of annuli on the 36th vertebra also seems to be difficult after the 8th annulus.

Otoliths

The mean width of the terminal translucent zone for female western Atlantic giant bluefin tuna captured in January through October 1975-81 indicated that May, June, and October were the months of slowest growth (Fig. 5), although slow growth was also observed during other months. Differences between monthly median terminal translucent measurements were not significant ($\alpha < 0.05$) based on Kruskal-Wallis analysis of either the long or short arms of sectioned sagittae. The occurrence of larval bluefin tuna in plankton net samples (Richards 1976) was closely associated with peaks of slow growth during the spring (Fig. 5). The peak of slow growth in the fall (October) was not associated with reproduction and appears to be related to fall offshore movement from the northeast coast and migration to overwintering grounds (Butler 1971; Rivas 1976).

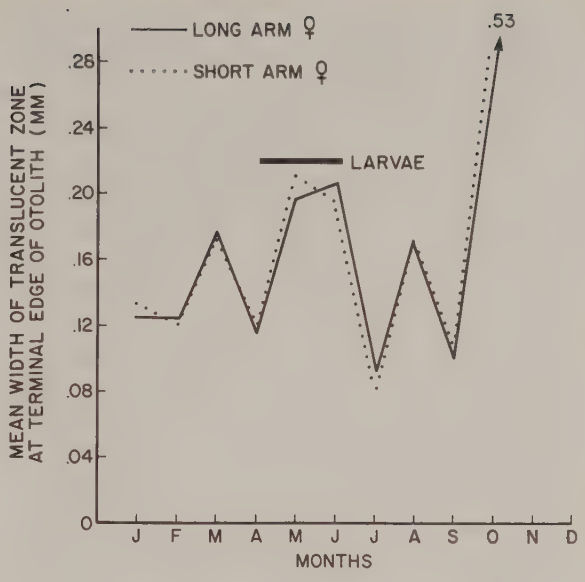

Figure 5.—Seasonal growth derived from otoliths of western Atlantic bluefin tuna sampled between 1975 and 1981. The seasonal occurrence of bluefin larvae in plankton net samples from the Gulf of Mexico as taken from Richards (1976).

Comparison of Otolith and Vertebrae Ageing Methods

Six pair-wise regressions (Table 1) between the four hardpart counting methods showed that estimated ages of all methods were significantly different from each other at $\alpha < 0.05$ (i.e., none of the slopes were equal to unity). However, the comparisons between counts on the 35th and 36th vertebrae and between the 35th vertebra and revised otolith were not significantly different at $\alpha < 0.01$. Mean absolute differences clearly show that the total otolith count is very different from the other three estimates of age. No relationship was found between the estimated ages of the four counting methods and fork lengths of the 20 tuna ($r^2 < 0.10$ for all methods vs. fork length).

Comparison of the Four Ageing Methods and Other Studies

Comparisons between the four methods of counting annuli and the length-at-age relationships developed in nine other studies (Table 2) indicated the following: 1) Estimated age from the 36th vertebra and the growth relationship from vertebrae and length frequency analyses of Bard et al. (1978) had the best agreement; 2) the second best agreement was with age estimates from otoliths and the growth curves from otoliths (Butler et al. 1977; Hurley et al. 1981); 3) the growth curves derived from tagging studies had the poorest agreement; 4) overall, the 35th and 36th vertebrae and revised otolith count performed about equally well, and the total otolith count consistently overestimated actual length of fish, except when compared with growth curves derived from otolith studies; and 5) in general, all age comparisons were poor as evidenced by standard deviations being 3-4 times the mean difference in lengths when this mean was ≤ 10 cm.

Table 2. — Mean difference (length in cm) and standard deviation (in parentheses) for length-at-age comparisons between nine bluefin tuna growth studies (via von Bertalanffy growth equations) and four ageing methods from 20 giant tuna.

Study	Ageing methods			
	Vertebrae		Otoliths	
Method of ageing	35th	36th	Total	Revised
Parrack and Phares (1979)	12.3	24.4	-16.9	13.1
tagging	(15.6)	(16.1)	(23.3)	(18.6)
Farber and Lee (1981)	-37.1	-21.0	-77.6	-36.0
tagging	(18.8)	(19.3)	(31.3)	(23.1)
Hurley et al. (1981)	11.5	20.2	-5.3	12.3
otoliths (female)	(13.2)	(14.1)	(16.0)	(15.3)
Butler et al. (1977)	17.4	27.1	-3.2	18.2
otoliths (female)	(13.8)	(14.5)	(18.0)	(16.1)
Farrugio (1980)	-8.5	9.8	-64.0	-7.7
vertebrae (36th)	(21.0)	(20.6)	(42.0)	(25.6)
Bard et al. (1978)	-9.2	2.6	-34.9	-8.2
vertebrae and length frequency	(15.2)	(16.0)	(21.3)	(18.2)
Rodriguez-Roda (1971)	-19.4	-5.5	-52.8	-18.4
vertebrae (4th & 5th)	(16.9)	(17.5)	(26.3)	(20.6)
Mather and Schuck (1960)[1]	-9.2	7.7	-57.2	-8.3
vertebrae (caudal), scales, length frequency	(19.7)	(19.6)	(36.5)	(24.0)
Mather and Jones (1972)[2]	-10.4	6.8	-59.4	-9.5
length frequency	(19.9)	(19.8)	(37.2)	(24.2)

[1]Von Bertalanffy growth relationship computed by Sakagawa and Coan (1974).
[2]Mather, F. S., III, and A. C. Jones. 1972. A preliminary review of the stock structure of bluefin tuna in the Atlantic Ocean. Unpubl. manuscr., 18 p. Woods Hole Oceanogr. Inst., Woods Hole, MA 02542.

DISCUSSION

Relationship Between Size of Fish and Size of Hardpart

An important assumption inherent in growth studies using skeletal hardparts is that size of fish and size of hardpart are closely related throughout the entire life cycle (Watson 1967; Lagler 1970; Smith 1983). Although many attempts have been made to estimate the age of western and eastern Atlantic bluefin tuna using skeletal hardparts, only rarely have studies examined this relationship for the purposes of back calculation. Rodriguez-Roda (1964) and Farrugio (1980) found significant relationships between vertebral cone radius and fork length of bluefin tuna ($r = 0.99$ in both studies). These analyses involved the entire size range of fish available (up to 275 cm FL), and giant-size specimens had relatively small sample sizes and were not analyzed separately.

The problems related to determining age and growth of giant bluefin tuna are illustrated by our regression analyses between size of bluefin tuna and size of their vertebrae and otoliths. As bluefin tuna reach the giant-size category, the relationship between the size of both hardparts and fork length deteriorates. The linear relationship between vertebral cone radius and fork length indicates a better fit than either the linear or curvilinear relationship between size of otolith and fork length. In addition, a significant linear relationship between vertebral cone radius and fork length was found for all size classes (including giants), whereas there was a breakdown in this relationship for otoliths ($r^2 = 0.10$) in giant bluefin tuna (accounting for the curvilinear fit). Although some deterioration of these relationships was expected when analyses were conducted on partial size categories of the entire data set, the significance level of these relationships is the important statistical indicator. Therefore, the linear fit for vertebrae will provide a more accurate back-calculated estimate of length for giant bluefin tuna than the curvilinear fit for otoliths. Accordingly, vertebrae should be used rather than otoliths in growth studies of bluefin tuna using back-calculated lengths from hardparts.

Growth Bands on Vertebrae

The return of tag 01171 extends the at-large tag-recapture data for Atlantic bluefin tuna from 14 yr (Mather 1980) to 15.8 yr. The 36th vertebra obtained from this fish represents the first opportunity to validate age estimates of giant bluefin tuna based on a skeletal hardpart. Previous reports of giant bluefin tuna tagged as school-size fish (< 5 yr old) and recaptured after 10 or more years at liberty (Mather 1980) indicated that fish caught in the summer with recapture lengths of 251 and 256 cm FL weighed 329 and 397 kg, respectively. Sexes of these recaptured fish are unknown. The estimated lengths for tag return 01171 (254 and 257 cm FL) closely approximate those reported by Mather (1980), but the recapture weight of 224 kg was more than 100 kg less than other tag returns of about equal length, which suggests this fish might be a female. This disparity in weight could be a result of the Bahamas specimen being an unusually slow-growing individual, the effect of spring vs. summer growth pattern, or differentiated growth between sexes. However, previous reports by Rivas (1976), Butler et al. (1977), Hurley et al. (1981), and Hurley and Iles (1983) indicate that female giant bluefin tuna weigh less, on the average, than males at similar lengths.

The predicted lengths for an 18-yr-old from Parrack and Phares (1979), females from Butler et al. (1977), and females from Hurley et al. (1981) were 256.2, 239.5, and 260.3 cm FL, respectively. Since our most reliable estimate of length at capture (257 cm FL) falls within 1 cm of that predicted by Parrack and Phares (1979), we agree with the conclusion of Mather (1980) that this relationship is accurate for giant bluefin tuna. Therefore, our study supports continued use of the Parrack and Phares (1979) length-at-age relationship for stock assessment, until a better alternative becomes available.

The tagging data used to determine the age of the giant bluefin tuna caught in the Bahamas appear reliable (Hurley and Iles 1982). If the reported size at tagging is assumed to be accurate, then previous data on early growth (Parrack and Phares 1979, and others) indicate virtually no chance that the fish was short enough in fork length to be 1+ yr old and only a slight chance that the fish could have been long enough to be in its 3rd year of life. However, it was not clear from the tagging records whether this fish was actually measured or size was estimated on the basis of average length from that particular catch or school (Hurley and Iles 1982). Overestimates of length at tagging could account for the 1-yr difference observed between age based on tagging records (18+ yr) and revised age estimated from the 36th vertebra (17+ yr). We feel an error of this type is remote since average lengths between 1- and 2-yr-olds do not overlap (Westman and Gilbert 1941; Mather and Schuck 1960; Parrack and Phares 1979), and this distinction would have been obvious, even in a field situation. Mather (1980) also recognized the possibility of error related to estimated lengths of tagged school-size fish, but concluded these errors are minor and were not sufficient to affect estimated age. Therefore, the factors contributing to differences in age

determination of the tagged fish appear to be related to problems of distinguishing, counting, and measuring growth bands, not errors related to estimates of age at tagging.

Numerous studies have established the relative ease of determining age of young bluefin tuna ($\leq$ 5 yr) from length frequency analysis, scales, otoliths, and vertebrae (Westman and Gilbert 1941; Mather and Schuck 1960; Berry et al. 1977; Parrack and Phares 1979; Farber and Lee 1981). Our interpretation of early growth bands (1-5) on the 36th vertebra was in close agreement with focus to annuli measurements on the 35th vertebra for 1,029 bluefin tuna (Fig. 4). Thus, interpretation of early growth on the vertebra from the tagged fish appears to be correct and suggests that if errors were made, they probably occurred as counts of increments progressed towards the centrum margin. In addition, our analysis demonstrated that the difficulty of distinguishing between the closely spaced increments on the centrum margin was acute. This problem starts after the 5th annulus and becomes severe at about the 8th, 9th, or 10th annulus (Fig. 4). Changes in patterns of growth related to reproduction (i.e., mature vs. immature growth discussed by Pannella 1980) appear to be related to this problem since Baglin (1982) found that most western Atlantic bluefin tuna were spawning by ages 8-10. Although similar problems of distinguishing and counting growth increments on vertebrae after the 8-10th annuli have been reported by others (Mather and Schuck 1960; Rodriguez-Roda 1964; Caddy and Butler 1976), the reasons for it have not been addressed.

Other attempts to age bluefin tuna using vertebrae have been unable to establish the interpretation of the crowded increments on the centrum margin (Mather and Schuck 1960; Rodriguez-Roda 1964; Caddy and Butler 1976). Since the revised estimate of age based on the vertebra from the tagged fish indicates an underestimate of 1 + yr, it appears that these bands should be interpreted as equal to ***at least*** 1 yr each.

Growth Bands on Otoliths

Metabolic changes associated with migration and reproduction have been reported to contribute to periods of slow growth and annulus formation in bluefin tuna (Rivas 1954; Tiews 1963; Butler et al. 1977; Compeán-Jimenez and Bard 1980, 1983). Butler et al. (1977) speculated that slow growth and translucent zone formation on bluefin tuna otoliths occurred during December to May. However, they also acknowledged the occurrence of peripheral translucent bands on otoliths from some fish in Canadian waters in late fall. The peaks of seasonal slow growth we observed in the spring and fall correspond well with the migration and reproduction of western Atlantic bluefin tuna. This suggests that giant bluefin tuna could be depositing two or more bands on their otoliths each year after age at first spawn, which is believed to be about age 10 for females (Baglin 1982). Although we felt these data were strong enough to warrant examination of this hypothesis, our two bands per year approach should be tempered by the assumption that translucent characteristics represent slow growth (Brothers[3] reports this is not always the case) and the lack of statistical difference found in monthly translucent zones.

Our revised otolith method of ageing (counting two bands each year after the 10th increment) closely approximated results of the 35th vertebra estimates for the 20 female giant bluefin tuna examined (Table 1). In addition, the revised otolith method performed equally well with the other methods in fitting length-at-age relationships of previous studies (Table 2). Therefore, interpreting two annual growth bands per year after the 10th band on giant bluefin tuna otoliths appears reasonable and has a strong biological rationale, although we were not able to definitively test this hypothesis. Thus, we believe this interpretation warrants further investigation.

Length at Age

The lack of a relationship between fork length and estimated age based on vertebrae and otoliths of the 20 giant bluefin tuna indicate that this relationship may be unreliable for use in age and growth studies, no matter what ageing method is used. The occurrence of a greater proportion of slow- and fast-growing individuals in giant-size fish or the relatively small sample size of our study may contribute to the lack of correlation between fork length and age compared with younger bluefin tuna, but the reason(s) for this remains unknown.

The degree of agreement between length-at-age comparisons of the four ageing methods and relationships developed in nine other studies (Table 2) generally reflected the method of ageing used in each. For example, the 36th vertebra counts came close to predicting length-at-age from Bard et al. (1978) based on vertebrae and length frequency analyses, whereas total otolith counts reflected previous studies based on otoliths (Butler et al. 1977; Hurley et al. 1981). An exception to this trend was the revised otolith method, which came closest to predicting length-at-age for studies based on scales, vertebrae, and length frequency analysis. The fact that the revised otolith method performed about equally well compared with other methods leads us to conclude that the hypothesis of two annuli per year after age 10 cannot be rejected at this time. In addition, all age comparisons were relatively poor as evidenced by the large standard deviations of mean differences (Table 2), and few if any additional conclusions can be made.

Many of the studies examined had relatively small sample sizes for giant-size bluefin tuna and none of the previous works offered conclusive validation of their age estimates for giants. Therefore, a more detailed assessment on the accuracy of these studies and those methods used in this study cannot be made. Overall, these analyses indicate that many of the problems related to ageing giant bluefin tuna remain, for the most part, unresolved.

SUMMARY

1) The linear relationship between vertebral cone radius and fork length was stronger than either linear or curvilinear relationships between otolith length and fork length. This suggests that vertebrae should be used rather than otoliths in growth studies when back-calculated estimates of length are made from hardpart measurements.

2) The tag return of a giant bluefin tuna recaptured in the Bahamas after 15.8 yr extends the tag-recapture at-large data by almost 2 yr. The 36th vertebra obtained from this fish represents the first opportunity to validate vertebrae estimates of age for giant bluefin tuna.

3) All growth increments on vertebrae, including the closely spaced bands on the centra margin of giant bluefin tuna, should

[3]Brothers, E. B., Assistant Professor, Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, NY 14853, pers. commun. 30 December 1981.

be interpreted as equal to ***at least*** 1 yr each based on our analysis of the 36th vertebra from one giant bluefin where age was known from tagging records.

4) Early growth bands on the 36th vertebra (1-5) were in close agreement with mean focus to annuli measurements on the 35th vertebra of over 1,000 bluefin tuna and indicate correct interpretation. Thus, any errors in estimating age of this fish are probably due to miscounting the closely spaced increments on the centrum margin after annuli 8-10.

5) The length-at-age of the tagged fish fell almost directly on the Parrack and Phares (1979) growth curve and supports continued use of this relationship for stock assessment.

6) The weight at capture for the tagged fish was more than 100 kg lighter than previous recaptures of similar length and could be a result of unusually slow growth, the effect of a spring vs. summer growth pattern, or differentiated growth between sexes.

7) Migration and reproduction aspects of western Atlantic bluefin tuna life history correspond well with presumed slow growth as determined by terminal translucent zone measurements of sectioned sagitta and suggest at least two major slow-growing periods between January and October for giant bluefin tuna after age at first spawn.

8) The revised otolith method of ageing when compared with other ageing methods performed equally well in agreeing with length-at-age relationships of previous studies. Thus, the hypothesis that two annuli are deposited on otoliths each year after age 10 cannot be rejected at this time.

9) The lack of a relationship between fork length and age of the 20 giant bluefin tuna indicate that length-at-age relationships for giants may be unreliable, no matter what ageing method is used.

10) Of the four methods of age determination examined, the 35th vertebra and the revised otolith count appear to give the most accurate estimates of age. The 36th vertebra count tends to underestimate age and the total otolith count seems to overestimate age.

11) Present estimates of age for giant Atlantic bluefin tuna are not validated (except for the vertebra example given above), and thus the problem of ageing giants remains, for the most part, unresolved.

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Age and Growth Estimation of Atlantic Bluefin Tuna, *Thunnus thynnus*, Using Otoliths

PETER C. F. HURLEY and T. DERRICK ILES[2]

ABSTRACT

Age and growth were estimated by examining otolith sections from 1,416 Atlantic bluefin tuna, *Thunnus thynnus*, collected in the northwest Atlantic from 1975 to 1981. Individual length-at-age data were fitted to the von Bertalanffy growth curve and produced estimates for L_∞, k, and t_0 of 278 cm FL, 0.17, and 0.25 yr for males, and 266 cm FL, 0.17, and 0.11 yr for females, respectively. Our estimates of L_∞ are lower and of k higher than those reported in other ageing studies on bluefin tuna.

INTRODUCTION

Recent studies have indicated a serious decline in abundance of the Atlantic bluefin tuna, *Thunnus thynnus*, particularly in the western Atlantic (Caddy and Butler 1976; Parrack[1] 1980, 1981, 1982; Hurley et al. 1981; Hurley and Iles 1982a). Because the Atlantic bluefin tuna supports valuable commercial and recreational fisheries, a suitable method of age determination is necessary to insure effective management.

A wide variety of ageing techniques have been applied to Atlantic bluefin tuna, including modal analysis of length frequencies, tagging studies, and examination of hardparts such as scales, vertebrae, otoliths, and dorsal fin spines (Sella 1929; Westman and Gilbert 1941; Mather and Schuck 1960; Butler 1971; Rodriguez-Roda 1971; Caddy and Butler 1976; Nichy and Berry 1976; Berry et al. 1977; Butler et al. 1977; Bard et al. 1978; Parrack and Phares 1979; Mather 1980; Compeán-Jimenez and Bard 1980, 1983; Farrugio 1980; Farber and Lee 1981; Hurley et al. 1981; Lee et al. 1983). While many studies produced comparable results in ageing younger bluefin tuna, those using length frequencies, scales, and vertebrae reported difficulty in estimating the age of older bluefin tuna. This problem was also recently addressed by Lee et al. (1983).

It was only recently that otoliths were used for estimating the age of giant (>210 cm fork length, FL) bluefin tuna (Nichy and Berry 1976; Caddy and Butler 1976). Butler et al. (1977), using otoliths from 189 giant bluefin tuna taken in Canadian waters during 1975 and 1976, estimated ages ranging from 13 to 30 yr. They fitted these data to the von Bertalanffy growth curve and obtained estimates of L_∞, k, and t_0 of 287 cm FL, 0.13, and −0.33 yr for males and 277 cm FL, 0.12, and −0.80 yr for females, respectively. Hurley et al. (1981) expanded this data set by continuing to sample bluefin tuna in Canadian waters from 1977 to 1979 and obtained similar results using 1,095 giant bluefin tuna (Table 1).

Butler et al. (1977) and Hurley et al. (1981) sampled only giant bluefin tuna and used weighted mean length-at-age data for ages 1 to 4 from Mather and Schuck (1960), in order to obtain a fit to the von Bertalanffy growth curve. Our objective was to further expand this data set by sampling smaller fish, so that data would be available over the entire age range to obtain a more accurate otolith estimate of Atlantic bluefin tuna age and growth.

Table 1.—Parameter estimates of the von Bertalanffy growth curve for Atlantic bluefin tuna from various sources.

Source	Parameters		
	L_∞	k	t_0
Rodriguez-Roda (1971)	356	0.09	−0.89
Sakagawa and Coan (1974)			
from Mather and Schuck	437	0.06	−1.49
from Mather and Jones	447	0.05	−1.59
Butler et al. (1977)			
males	287	0.13	−0.33
females	277	0.12	−0.80
Bard et al. (1978)	318	0.11	−0.62
Parrack and Phares (1979)	313	0.09	−0.96
Compéan-Jimenez and Bard (1980)	370	0.07	−1.58
Compéan-Jimenez and Bard (1983)	372	0.07	−1.71
Farrugio (1980)	351	0.08	−1.09
Farber and Lee (1981)			
from mark-recapture data	313	0.12	−0.14
from vertebrae data	401	0.08	−0.92
Hurley et al. (1981)			
males	281	0.15	0.05
females	271	0.14	−0.21
Present study			
males	278	.17	.25
females	266	.17	.11

MATERIALS AND METHODS

Giant bluefin tuna, taken by trap net and rod and reel in Canadian inshore waters from 1975 to 1981, were sampled to obtain otoliths for ageing studies. In addition, small- and medium-size bluefin (≤ 210 cm FL), taken by purse seine off the east coast of the United States in 1981 and landed in Canada, were also sampled for otoliths. All fish were caught between July and November. Fork length (straight line distance measured from the tip of the upper jaw to the fork of the tail) was measured using calipers, and sex was determined by

[1]Department of Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick, Canada; present address: Marine Fish Division, Fisheries Research Branch, Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2.

[2]Marine Fish Division, Fisheries Research Branch, Department of Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick, Canada E0G 2X0.

gross examination of the gonads. The techniques used to collect, section, and read the otoliths were essentially those described by Butler et al. (1977) and Hunt (1978). The terminology used here to describe structures of the sagitta otolith (see figure 1 of Lee et al. 1983) follows those recommended by Hunt (1978).

The otolith collection, preparation, sectioning, and reading techniques used were described by Butler et al. (1977). Alternating translucent (or hyaline, slow growth) and opaque (fast growth) bands were clearly visible (see Glossary), particularly on the distal part of the limbs (Fig. 1). Due to the problems associated with positively identifying the nucleus and the lack of an accepted standard, a line was drawn across the narrowest portion of the otolith limb. This line was assumed to represent the starting point for initiation of growth, and only the bands distal to this arbitrary baseline were considered. Band formation was considered clearest on the short limb (medial-dorsal ridge) of the otolith section and this limb was used for age estimation, as in Butler et al. (1977) and Hurley et al. (1981).

Butler et al. (1977) speculated that the relationship between growth bands observed in sections of sagittae otoliths and the life cycle of bluefin tuna was as follows: 1) Translucent band—slow growth period, laid down from December to May in subtropical and tropical waters that are usually characterized by high temperature (15°-25°C) and relatively low food availability, and 2) opaque band—fast growth period, laid down from June to November in temperate waters that are usually characterized by lower temperatures (5°-15°C) and an abundance of food. Following this interpretation, age was estimated as the number of translucent bands observed, but not including the peripheral band if one was present, as was the case with some fish caught in the fall.

Otolith readings were performed by at least two independent readers. If agreement between readers was not obtained, the second otolith was sectioned. The specimen was rejected if agreement could not be reached. The use of a camera lucida, used after 1978 as a reference aid for the reader, improved reader performance and reduced variations in the readings.

Individual length-at-age data were fitted to the von Bertalanffy growth model, separately for both sexes, using the method of Allen (1967). Other growth models were not used, and there is no suggestion that the von Bertalanffy is the most appropriate—the von Bertalanffy model was chosen solely for making comparisons with other studies.

RESULTS

Otolith age estimates were obtained for 1,416 Atlantic bluefin tuna; 953 males, and 463 females. Estimated ages ranged from 1 to 30 yr for males and 1 to 32 yr for females. However, the fish of maximum observed lengths of 300 cm FL for males and 297 cm FL for females were assigned estimated ages of 24 and 25 yr, respectively. Parameter estimates for the von Bertalanffy growth equation, with 95% confidence intervals, were:

	Males			*Females*		
		Lower	*Upper*		*Lower*	*Upper*
L_∞	277.805	276.037	279.573	266.431	264.172	268.689
k	0.169	0.160	0.179	0.170	0.156	0.185
t_0	0.254	0.049	0.460	0.106	-0.234	0.445

The range of observed length-at-estimated ages for bluefin tuna based on counts of sectioned otoliths averaged 64.0 cm FL for males and 64.5 cm FL for females at estimated age 1, to 273.7 cm FL for males at estimated age 30, and 268.0 cm FL for females at estimated age 32 (Table 2). The fits of the observed data to the von Bertalanffy growth model (Fig. 2) indicate a high degree of variability for males and females.

DISCUSSION

The results presented here indicate that males grow slightly faster than females and reach a slightly larger size. These trends were reported previously by Butler et al. (1977) and Hurley et al. (1981). The estimates of the von Bertalanffy growth parameters presented here are very close to those using otoliths reported by Butler et al. (1977) and Hurley et al. (1981). The effect of extending the data set to include small- and medium-sized fish and using individual length-at-estimated age data, compared with mean length-at-estimated age data, has been to produce slightly lower values of L_∞ and slightly higher values of k compared with other ageing studies on bluefin tuna (Table 1). Southward and Chapman (1965) demonstrated that the von Bertalanffy parameters k and L_∞ are affected by both the range and the distribution of data. We were unable to determine if this was a factor in producing lower L_∞ and higher k values in this study, since sufficient information regarding the range and distribution of data was not available for most other bluefin tuna ageing studies. The parameter estimates produced here were closest to those reported from studies using mark-recapture data, where long-term tag returns suggested ages up to 24 yr (Parrack and Phares 1979; Farber and Lee 1981), while they differed most from those reported from studies using length-frequency analysis, scales, vertebrae, and dorsal fin spines (Rodriguez-Roda 1971; Sakagawa and Coan 1974; Compeán-Jimenez and Bard 1980, 1983; Farber and Lee 1981).

Hurley and Iles (1982b) reported a 16-yr-at-large bluefin tuna tag-recapture and an age-at-recapture of 18 yr based on length-at-release data (Lee et al. 1983). Unfortunately, sex was not determined and otoliths were not available for validation purposes, but the 36th vertebra was obtained and analyzed by Lee et al. (1983). Using the estimated size-at-recapture of 257 cm FL, the von Bertalanffy parameter estimates calculated for this example would underestimate age-at-recapture by 3 yr (15 yr old), if the fish was a male, and overestimate age-at-recapture by 2 yr (20 yr old), if the fish was a female. Given the variability in the length-at-age estimates, no conclusions regarding either the fit of the model or the question of banding periodicity on otoliths can be reached based on this tagging data. However, Lee et al. (1983) were able to make more definitive conclusions in terms of banding periodicity on vertebrae, based on their analysis of these data.

The individual length-at-age estimates (Table 2) suggest a large degree of variability in growth rate. Lee et al. (1983) also reported highly variable growth rates of giant Atlantic bluefin tuna. This may be due to errors generated in the reading technique or may reflect real variability in the growth rate of this species. There are several possible sources of error in the reading technique:

1) There is difficulty in defining the nuclear region and the choice of an arbitrary starting point; however, this would

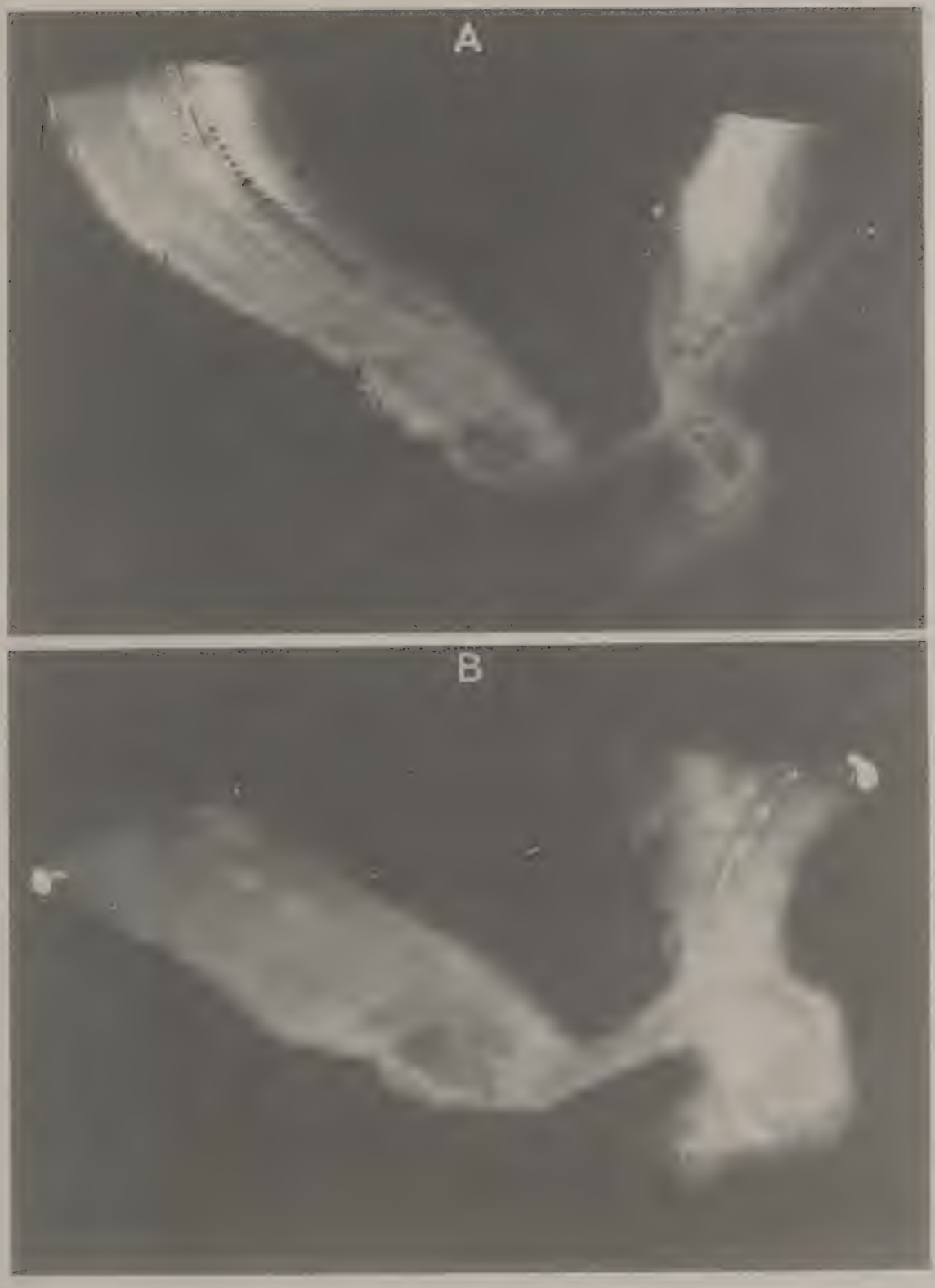

Figure 1. – Cross section of sagitta otolith of: A) A female Atlantic bluefin tuna 267 cm FL, estimated age 29 yr, and B) a female Atlantic bluefin tuna 141 cm FL, estimated age 5 yr.

Table 2.—Mean observed fork length (cm) at estimated age, standard deviation (SD), and number of observations for male and female Atlantic bluefin tuna based on counts of sectioned otoliths.

	Males			Females		
Estimated age (yr)	Mean observed fork length (cm)	SD	No. of observations	Mean observed fork length (cm)	SD	No. of observations
1	64.0	1.00	3	64.5	—	1
2	75.5	9.10	7	82.3	4.17	4
3	77.3	13.79	2	82.5	7.42	2
4	113.3	15.45	5	130.0	20.06	4
5	147.1	9.03	10	149.4	22.07	8
6	146.4	20.94	4	161.1	15.38	12
7	173.3	37.29	3	175.0	28.28	2
8	253.0	—	1	—	—	—
9	223.7	13.58	3	—	—	—
10	234.0	—	1	—	—	—
11	234.5	11.54	7	229.0	12.73	2
12	234.5	4.95	2	262.0	—	1
13	256.3	12.27	5	251.5	9.19	2
14	253.5	12.81	11	252.0	14.14	2
15	262.9	11.39	20	250.3	11.87	4
16	262.5	10.76	31	250.1	7.09	5
17	262.5	10.51	53	252.5	8.97	22
18	264.0	11.36	71	258.9	13.60	25
19	267.5	11.67	126	258.0	10.06	38
20	268.7	9.66	94	259.3	12.27	39
21	268.3	10.09	102	257.4	10.58	49
22	270.4	9.12	129	259.8	8.91	62
23	270.1	7.92	105	259.1	9.52	40
24	271.5	10.58	68	260.3	10.32	48
25	272.2	9.81	45	260.6	11.41	34
26	274.4	9.13	23	262.7	9.59	24
27	274.4	10.44	12	265.0	12.28	13
28	272.5	7.77	4	256.6	7.79	5
29	272.7	4.04	3	259.0	7.52	5
30	273.7	5.60	3	271.1	13.08	5
31	—	—	—	265.7	7.51	3
32	—	—	—	268.0	5.66	2

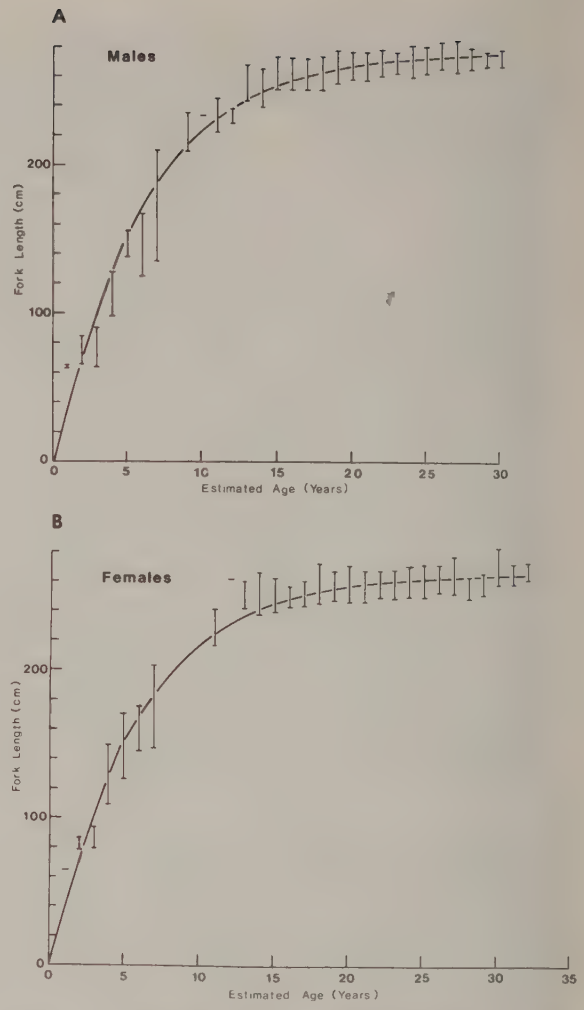

Figure 2.—Von Bertalanffy growth curve from length-at-age data for: A) Male, and B) female Atlantic bluefin tuna. Brackets indicate one standard deviation on either side of mean, dashes indicate individual observations.

likely produce a consistent error, not the wide variability observed.

2) There is also difficulty in distinguishing annuli (see Glossary), i.e., the annuli are relatively evenly spaced and distinct distal to about the 10th annulus but the proximal annuli, particularly the region of the 1st to 5th annuli, are much less distinct and are a possible source of error. Also, false checks may possibly be misread as annuli. The close agreement between readers suggests these errors are minimal but does not necessarily eliminate them.

3) The occurrence of sub-annular banding or changes in the pattern of band deposition during the life history may also cause error. Compeán-Jimenez and Bard (1983) reported that bluefin tuna from the eastern Atlantic deposited two bands per year in dorsal fin spines, each band corresponding to a seasonal migratory pattern. Lee et al. (1983) discussed in detail the possibility of changes in the pattern of band deposition during the life history of bluefin tuna in the western Atlantic, but could not conclude whether more than one band is deposited each year in otoliths. If changes in the pattern of deposition do occur, this would produce a consistent error and would increase variability in the growth rate.

Disregarding the peripheral translucent band in the few cases in which it occurred in this study may also represent a source of variability. It was not counted on the premise that bluefin tuna in this study were sampled between July and November and that a peripheral translucent band would represent an early onset of slow growth hypothesized to occur between December and May. This decision was made since samples were not available beyond November and that the correct cohort would be identified in this manner. The occurrence of a peripheral translucent band may lend support to the suggestion of more than one translucent band per year as Lee et al. (1983) suggest, but sampling over more of the year was required to attempt a meaningful analysis of terminal band width. Lee et al. (1983) obtained otolith samples over most of the year (except November and December), but were unable to draw definitive conclusions on whether more than one band was deposited per year based on a study of otolith terminal band width.

The high degree of variability in growth rate observed in our study, and reported by others, appears to be real and would be a result of one, or a combination of, the following factors of the biology of this species:

1) At such a rapid growth rate, especially up to about age 10, small changes in individual growth rates could produce significant differences in relative growth between individuals.
2) A large seasonal component of growth rate could generate variability in the results if samples are collected over the period of rapid growth. Butler et al. (1977) reported that giant bluefin tuna increase approximately 10% in body weight per month during the 5-7 mo spent per year in Canadian inshore waters.
3) Migratory patterns in bluefin tuna change with age and appear to have changed over time, as demonstrated by the collapse of local fisheries such as the Wedgeport, Nova Scotia, and Newfoundland rod-and-reel fisheries. Changes in temperature regime and food supply may produce changes in growth rate between cohorts.
4) The likelihood of substantial differences in individual growth rates increases as lifespan increases.
5) The intermixing of more than one stock, as suggested by tagging data, each with different growth rates, could contribute to variability in estimates of growth rate.
6) Recent stock assessments (Parrack 1980, 1981, 1982) have indicated substantial declines in stock abundance over the last 20 yr, which might result in changes in growth rates. If the age estimates in this study are accurate, cohorts from 1949 to 1980 (estimated ages 1-32) are represented in the study.
7) Long-term trends in the environment, in either temperature regime or food availability and abundance, could contribute to changes in growth rate between cohorts.

The results of this study are preliminary and little can be concluded other than this species appears to be long-lived and their growth rate is variable. Further study is required, particularly in the area of age validation (see Glossary). Analysis of hardparts from tagged fish of known size, age, and sex at both release and recapture or from tetracycline marking experiments would provide significant advances in bluefin tuna age assessment.

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Growth Increments on Dorsal Spines of Eastern Atlantic Bluefin Tuna, *Thunnus thynnus*, and Their Possible Relation to Migration Patterns

G. COMPEÁN-JIMENEZ[1] and F. X. BARD[2]

ABSTRACT

The first dorsal spine was taken from 227 bluefin tuna, *Thunnus thynnus*, caught in the Bay of Biscay, the Canary Islands, and the Mediterranean Sea during 1978 to 1979 to estimate age and growth rate, and to determine if a relationship exists between the growth increments registered on sections of the spines and fish migration patterns. Growth bands were not present in spines from young-of-the-year (40-44 cm fork length, FL), but first appeared in spines from tuna with an estimated age of 1+ yr (60-67 cm FL). For bluefin tuna < 3 yr old (60-89 cm FL), back-calculated fork lengths show that growth bands (consisting of one translucent and one opaque zone) are formed twice a year, except for the first band, which appears after the first growing season. The formation of these bands seems to be related to their spring and fall migration in the eastern Atlantic area. In addition, this general pattern of growth (two bands or one couplet per year after the first year) also seems to apply to our sample (139) of larger fish (100-280 cm FL).

The growth curve calculated for bluefin tuna closely approximates previous estimates of growth reported for the eastern Atlantic Ocean and Mediterranean Sea. The growth bands on dorsal spines are easily distinguished, particularly on young fish, and provide a simple, rapid method for estimating age and growth rates.

RESUMEN

La primera espina dorsal fué colectada de 277 atunes aleta azul, *Thunnus thynnus*, capturados entre 1978 y 1979, en el Golfo de Vizcaya, las Islas Canarias y el Mar Mediterráneo. Las muestras fueron utilizadas para estimar la edad, la tasa de crecimiento y la existencia de una posible relación entre las marcas de crecimiento en las espinas y los patrones de migración. No se observaron bandas de crecimiento en las espinas de juveniles del mismo año (40-44 cm de longitud horquilla, Lh), pero una primera banda aparece en las espinas de atunes con una edad estimada de 1+ años (60-67 cm Lh). En jóvenes atunes de menos de 3 años (60-89 cm Lh), el retrocálculo muestra que las bandas de crecimiento (formadas por una zona traslúcida y una zona opaca) se forman dos veces por año, excepto para la primera banda que aparece después del primer período de crecimiento. La formación de estas bandas parece estar relacionada con las migraciones de primavera y de otoño de los atunes jóvenes en el área del Atlántico Oriental. También, en general este patrón de crecimiento (dos bandas o un par por año-después del primer año) puede aplicarse a nuestra muestra (139) de peces adultos (100-280 cm Lh).

La curva de crecimiento calculada, para el atún aleta azul, se aproxima bastante a estimaciones previas que han sido reportadas para el Atlántico Oriental y el Mar Mediterráneo. Usando las espinas dorsales para estimar la edad se tiene la ventaja de un muestreo fácil y bandas de crecimiento muy claras particularmente en los peces jovenes.

INTRODUCTION

Many studies have reported the occurrence of incremental growth marks on skeletal hardparts of bluefin tuna, *Thunnus thynnus*, but these reports have rarely related the observed growth marks to environmental or behavioral aspects in the life of the fish (Mather and Schuck 1960; Nichy and Berry 1976; Lee et al. 1983; Hurley and Iles 1983). As indicated by Farrugio (1979) and Cort (1979), interpretations of growth bands on skeletal hardparts have been particularly difficult once this species reaches adult size (≥ 200 cm fork length, FL). This problem has recently been extensively reviewed by Lee et al. (1983) and Hurley and Iles (1983). Age estimates of giant bluefin tuna vary according to the method of ageing applied to the samples, the geographical location of the fish at capture, sample size, length distribution of samples, and the separation and analysis of samples by sex.

In contrast to ageing adult (giant) bluefin tuna, research results on juveniles (up to 50 cm FL) have been more consistent between studies and easier to document. For example, tuna born in the Mediterranean Sea in May-June showed a rapid growth in weight of 800 to 1,000 g during the first 4 mo of life (Piccinetti and Piccinetti-Manfrin 1970). This rapid growth rate was also verified in rearing experiments in Japan (Bard and Le Gall 1979). As a result of this fast growth and a single, relatively short spawning season, well-defined size distributions correspond to early age classes. Thus, these cohorts can be traced for 1 to 3 or 4 yr with very little error in age estimates. This is well illustrated by Furnestin and Dardignac (1962) who followed the growth of bluefin tuna along the Atlantic coast of Morocco from 6 mo old to 3 yr of age. Their results indicated rapid growth of juvenile tuna after their arrival (from the Mediterranean Sea) near the Moroccan coast (October-November) and very slow or negligible growth from January to March of the following year. Maximum growth was reached thereafter between the end of May and the begin-

[1]Instituto Nacional de la Pesca, Centro de Investigaciones Pesqueras, A. Postal 976, Ensenada, Baja California, Mexico.

[2]Centre de Recherches Oceanographiques, Office de la Recherche Scientifique et Technique Outre-Mer, V18 Abidjan, Ivory Coast.

ning of September, and slower growth was evident again in the fall when fish averaged about 63 cm FL or at the end of their first year of life. From information such as this, a cohesive pattern of migration and general life history of young, eastern Atlantic bluefin tuna can be established (Fig. 1).

In the Mediterranean Sea, bluefin tuna spawn principally from the beginning of June through August (Arena 1979). However, the possibility of other spawning zones near the Sahara coast, the Canary Islands (Aloncle 1967), and in the Black Sea cannot be excluded though they have never been confirmed. The contribution from these zones, if they exist, would probably be of limited importance. The majority of Mediterranean fish leave the area by the Strait of Gibraltar during the fall of their first year (Rey 1979). The fact that part of the bluefin tuna population stays in the Mediterranean Sea is demonstrated by the presence of juveniles caught during the entire year (Farrugio 1977; Scaccini 1961). The bluefin tuna that leave the Mediterranean overwinter in Moroccan waters and some are captured at the beginning of the year by the purse-seine fleet stationed at Casablanca (Morocco). The next summer these bluefin tuna can be found in the Bay of Biscay, after their summer migration starting at the Moroccan coast (Bard 1977). At the end of the summer they return to their wintering waters in Ibero-Morocco Bay (Brethes 1979; Lamboeuf 1975). The adults (Fig. 2) are present during August in the offshore region of Norway. From there they return to southern waters by September-October (Hamre 1963) and reach their wintering zone along the south coast of Spain and the Canary Islands (Santos-Guerra 1976). The spawning migration to the interior of the Mediterranean Sea takes place during May to June. Right after spawning, the fish leave through the Strait of Gibraltar (Sella 1929; Rodriguez-Roda 1964, 1969; Sara 1973).

This information provides an opportunity to determine if a relationship exists between the growth increments on skeletal hardparts and the life history aspects of this species. Such hypotheses have been suggested in the past but conclusive information on the causes of growth band formation on hardparts are rare and have not been reported for eastern Atlantic bluefin tuna. Since Compeán-Jimenez and Bard (1980) found that well-defined growth marks were evident on the first dorsal

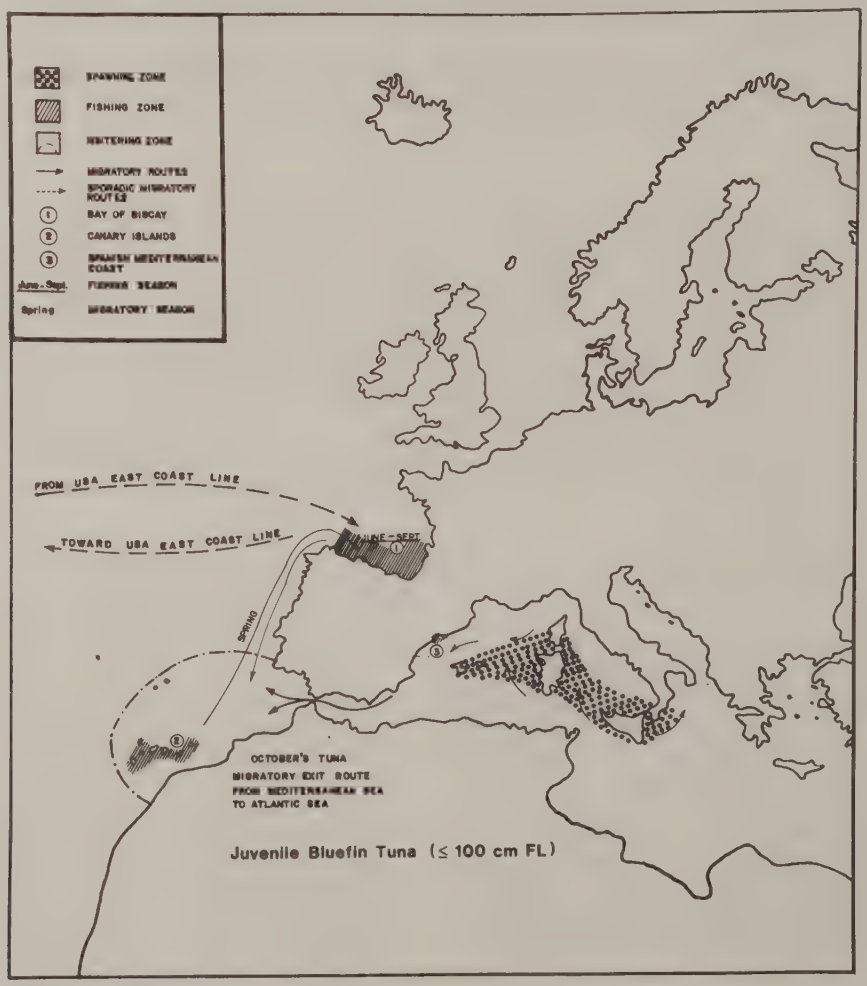

Figure 1.—Migration routes (solid lines) of juvenile bluefin tuna (≤ 100 cm FL) in the eastern Atlantic. Dashed lines (sporadic migration routes) represent general movements and direction (arrows) from data on tagged and recaptured tunas (they do not necessarily correspond to exact routes). Spawning zone (dots), fishing zone (slashed lines), wintering zone (dots and dashed lines) are shown for the Bay of Biscay (1), Canary Islands (2), and Spanish Mediterranean coast (3).

spines of bluefin tuna caught from the Atlantic, we chose the first dorsal spine as a source of age and growth information. Accordingly, the objectives of our study were to estimate the age and growth rate from growth bands on dorsal spine sections and to relate this information to the life history aspects of bluefin tuna in the eastern Atlantic Ocean.

METHODS

Bluefin tuna were collected from the bait-boat fishery in the Bay of Biscay, the handline fishery off the Canary Islands, and the trap fishery along the Spanish coast of the Mediterranean Sea (Fig. 3). Young bluefin tuna (< 100 cm FL) were sampled in the Bay of Biscay at the beginning of the fishing season in June and July and at the end of the season in September and November, 1978-79. A few giant bluefin tuna were also sampled in November 1978. Bluefin tuna > 100 cm FL were collected off the Canary Islands in March 1979 and a few young-of-the-year (40-44 cm FL) were also obtained in 1979 from the Mediterranean Sea.

The first dorsal spine was collected from each specimen together with measurements, such as fork length (cm) and total weight (kg), as well as date and location of capture. The spine extraction, sectioning, and preparation procedures generally followed those of Johnson (1983). Briefly, these procedures consist of making a cut (1.0 to 1.5 mm thick) near the condyle spine base (Fig. 4a) using a slow-speed saw. Three cross sections were taken from each spine, mounted on slides, and stored in boxes before reading.

Growth bands were counted on sections using transmitted light projected onto a screen through a microprojector (Fig. 4b). Typical growth patterns on bluefin tuna spines included a narrow translucent zone, which we assumed to be a slow-growth stage, and wider opaque zones which probably represent fast growth (Fig. 4b; also see Glossary). Details that support these assumptions appear in the Results section.

The following measurements were taken from each spine (Fig. 4b):

1) Spine diameter—the horizontal distance between the out-

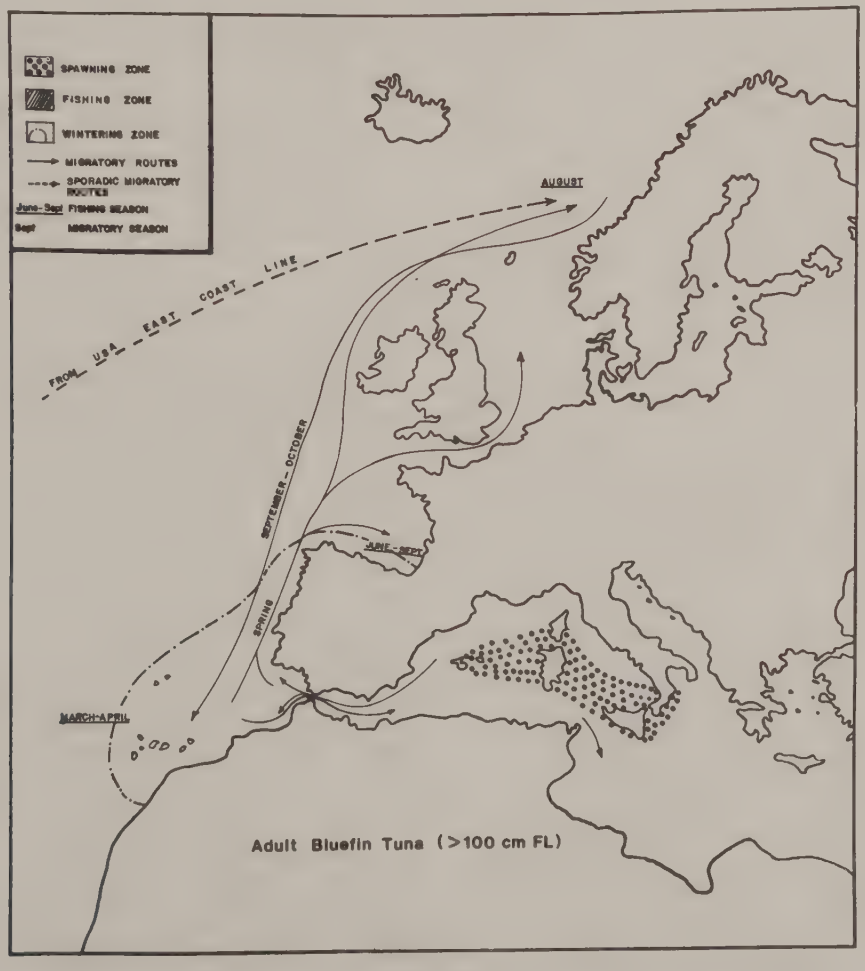

Figure 2.—Migration routes (solid lines) of adult bluefin tuna (> 100 cm FL) in the eastern Atlantic. Dashed lines (sporadic migration routes) represent general movements and direction (arrows) from data on tagged and recaptured tunas (they do not necessarily correspond to exact routes). Spawning zone (dots), fishing zone (slashed lines), wintering zone (dots and dashed lines) are shown for the Bay of Biscay (1), Canary Islands (2), and Spanish Mediterranean coast (3).

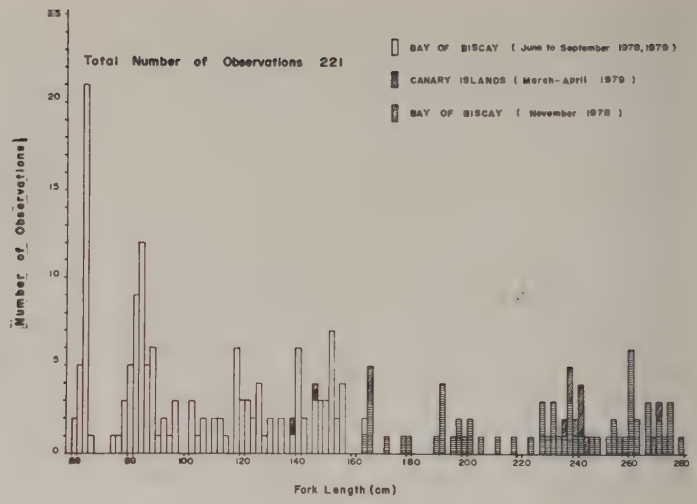
Total Number of Observations 221
BAY OF BISCAY (June to September 1978, 1979)
CANARY ISLANDS (March-April 1979)
BAY OF BISCAY (November 1978)
Number of Observations
Fork Length (cm)

Figure 3.—Length frequencies of tuna caught in the Bay of Biscay from June to September (clear bar), Canary Islands (horizontal slashed bar), and Bay of Biscay (angled slashed bar) in November.

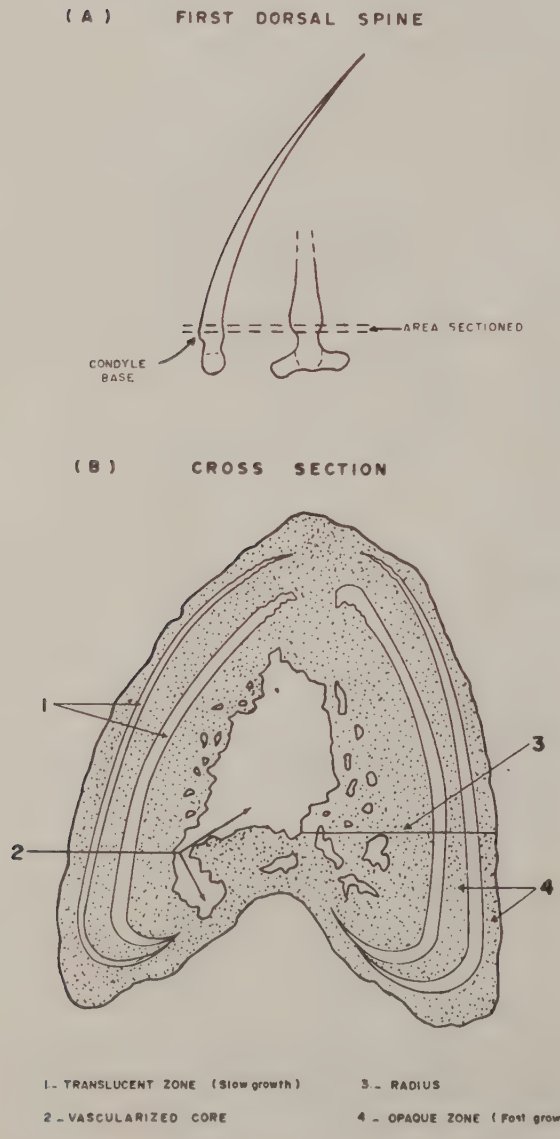

Figure 4.—Position of section removed from the first dorsal spine (A), and a cross section of this spine showing areas of zonation and measurements taken (B).

side margin above the posterior notch where the least curvature of banding occurred in each spine.

2) Spine radius—the distance (along the diameter) from the estimated center of the spine to the outside margin.
3) Growth increments—the distance along the diameter from the estimated center of the spine to the outside margin of each translucent zone.

The theoretical center of the spine, often obscured by the vascularized core, was estimated as a point one-half the diameter measurement inside the spine (Antoine et al. 1983). The relationship between spine diameter and fork length was determined with regression analysis. Statistical inferences were made with a significance level of $\alpha = 0.05$.

Growth band measurements were used as the basis for back calculating the size of fish at the time of band formation. In particular, the relationship between the size of young bluefin tuna (Bay of Biscay) and the location of their growth bands were used to establish the growth pattern for the first several years of life and to interpret growth bands in older bluefin tuna from the Canary Islands. Early growth bands on bluefin tuna 3 yr old and older were progressively obscured by the increasing size of the vascularized core as the size of the fish increased. Accordingly, the number of these obscured (lost) bands was estimated from observations of their position and number on younger specimens.

Estimated ages resulting from increment counts, measurements, and interpretations of growth bands were combined with fork lengths to construct a von Bertalanffy growth curve using the method of Abramson (1971).

RESULTS

Clearly defined rhythmic growth marks were observed on dorsal spine sections from almost all specimens < 89 cm FL and in most of the larger bluefin tuna. The marks appeared as either translucent or opaque zones (see Glossary) when viewed with transmitted light (Fig. 4b). Microradiographs (X-rays) of the translucent zones revealed hypermineralization, a charac-

teristic identified earlier by Meunier et al. (1979) as a slow-growth zone. There was a significant linear relationship between spine diameter and fork length (r^2 = 0.96, Fig. 5); therefore, we felt justified in using spine measurement to back calculate previous growth history.

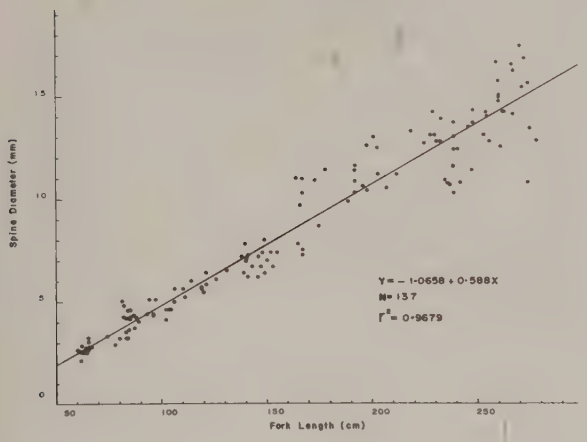

Figure 5.—Relationship between spine diameter (mm) and fork length (cm) of 137 eastern Atlantic bluefin tuna.

Mediterranean Sea

Six bluefin tuna (40-44 cm FL) were caught in October 1979, off the Castellon (Spanish) coast of the Mediterranean Sea, and examination of spine sections indicated no apparent growth marks or bands. Therefore, the reported spawning dates of May-June (Arena 1979) and the date of capture suggest that these fish were young-of-the-year or about 4-5 mo old. The fall season has been reported (Rey 1979) as the time of year when these young tuna make their first migration out of the Mediterranean Sea to Ibero-Moroccan Bay (Fig. 1).

Bay of Biscay

A total of 144 bluefin tuna (60-165 cm FL) were collected from the Bay of Biscay at the beginning of the fishing season in 1978 and at the end of the fishing season in 1978 and 1979 (Fig. 3). About half (78) of these specimens were < 100 cm FL. However, 66 larger fish (100-166 cm FL) were obtained from this area during the same period in 1979. A few giant bluefin tuna (14, > 200 cm FL) were also obtained in November 1978.

Examination of the Bay of Biscay samples revealed that the number of translucent (slow growth) bands increased between bluefin tuna with an estimated age of 1+ yr (60-67 cm FL) and an estimated age of 2+ yr (69-89 cm FL). Back calculations of the size and estimated age at band formation indicated, in general, that except for the first band, all others were formed in pairs or couplets each year (Figs. 6-9). The first band is well separated from the first complete couplet (Fig. 6), and information from young-of-the-year tuna from the Mediterranean Sea, as well as back calculations of fish from the Bay of Biscay, suggest this singular, first band is formed at about the 6th mo of life.

Figures 7-9 illustrate the progressive increase in the number of bands on spine sections from Biscay bluefin tuna with an estimated age of 1+ and 2+ yr. For example, 12 tuna sampled at the beginning of the season (Fig. 7) had one growth band with a back-calculated average size at band formation of 48.08 cm FL and a back-calculated date of band formation of October or November (6-7 mo after birth). Of the 17 tuna sampled at the end of that same fishing season (Fig. 7), 75% (12) had a second band (corresponding to the formation of the first part of the first couplet). The back-calculated average size at band formation of the second band was 55.25 cm FL, and the back-calculated date of band formation was August (about 1 yr 2 mo after birth). The 16 tuna with an estimated age of 2+ yr sampled at the beginning of the season (Fig. 8) had three distinct bands: One band formed after the first 6-7 mo of life and two bands formed during the following 12 mo. However, of the tuna sampled at the end of the season during the second year of life (Fig. 9), only 37% (9 of 24) had a fourth band. In addition, the first singular growth band, in four of these tuna, was obscured by the increased size of the vascularized core.

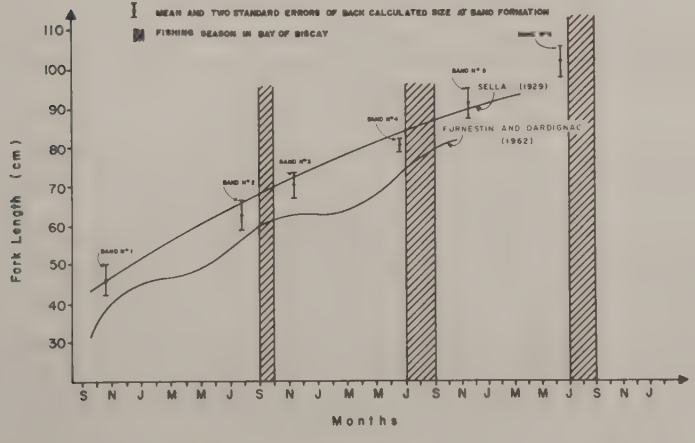
MEAN AND TWO STANDARD ERRORS OF BACK CALCULATED SIZE AT BAND FORMATION
FISHING SEASON IN BAY OF BISCAY
SELLA (1929)
FURNESTIN AND DARDIGNAC (1962)
Fork Length (cm)
Months
S N J M M J S N J M M J S N J M M J S N J

Figure 6.—Mean (± 2 SD) back-calculated fork lengths (cm) based on counts of bands (1-6) from spine cross sections of bluefin tuna caught in the Bay of Biscay, 1978-79. Fishing season in the Bay of Biscay is shown by slashed lines and growth curves of juvenile bluefin tuna estimated by Sella (1929, solid line) and Furnestin and Dardignac (1962, wavy line) are also shown.

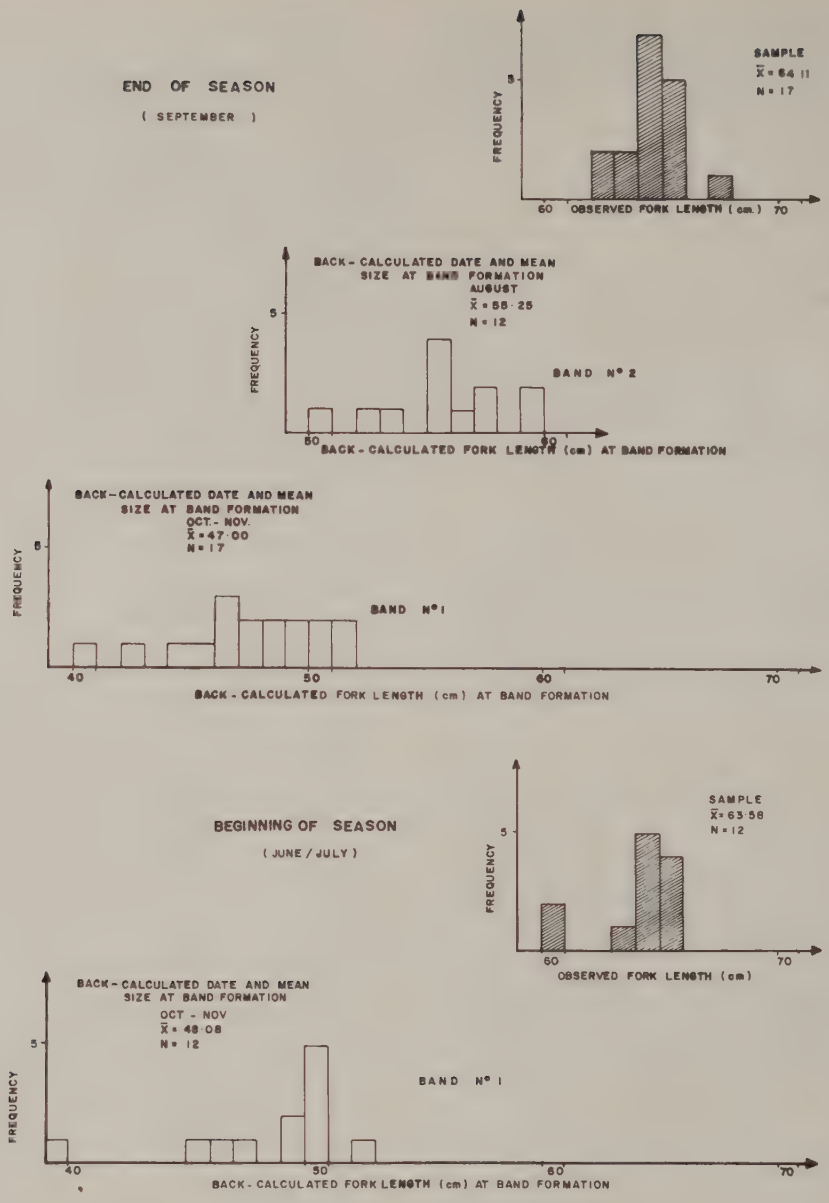

Figure 7.—Back-calculated date and mean size at band formation for bluefin tuna of estimated age 1+ from the Bay of Biscay at beginning of the season (N = 12) and at end of season (N = 17).

Canary Islands

A total of 63 bluefin tuna (164-280 cm FL) were collected from the fishery off the Canary Islands in 1979 (Fig. 3). Examination of spine cross sections indicated that the increased size of the vascularized core obscured early growth bands. In such cases, the method of estimating age is given by a detailed example in the paragraph immediately below. A maximum of 8 couplets or 16 bands were observed on spine sections of larger tuna. These data were combined with information from tuna collected in the Bay of Biscay to fit a curve to the von Bertalanffy growth model.

An example of the interpretation of growth bands applied to older fish is illustrated by back calculation of size-at-band-formation for a 201 cm FL specimen from the Canary Islands (Table 1). There were a total of 13 bands (6 couplets) plus the growth zone between the last band and the edge of the spine. Based on data from young bluefin tuna, the first visible couplet was judged to represent the end of the 4th year of life (seven bands were estimated to be lost due to the vascularized core,

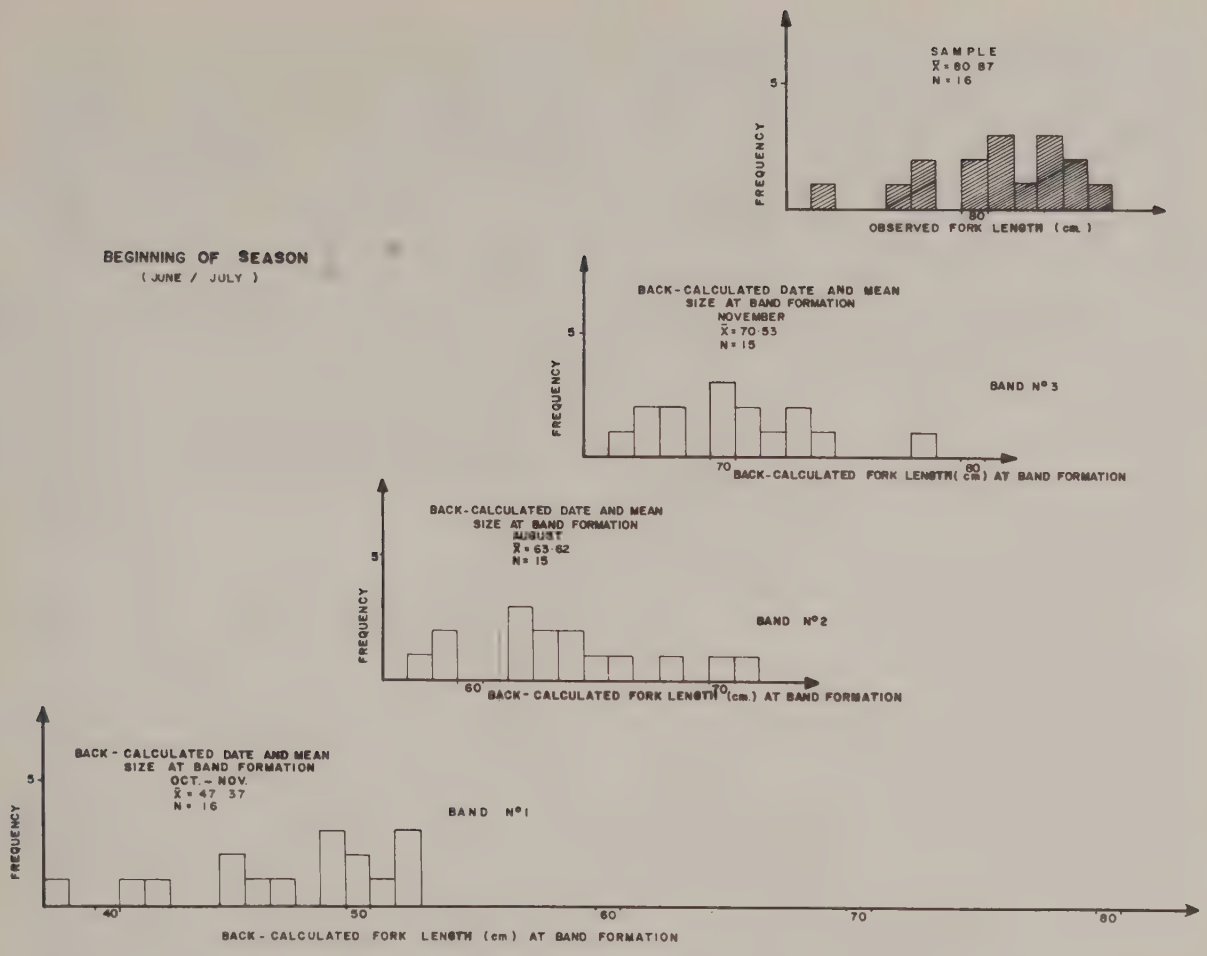
SAMPLE
X̄ = 80 87
N = 16
FREQUENCY
OBSERVED FORK LENGTH (cm.)
BEGINNING OF SEASON
(JUNE / JULY)
BACK-CALCULATED DATE AND MEAN SIZE AT BAND FORMATION
NOVEMBER
X̄ = 70 53
N = 15
BAND N° 3
BACK-CALCULATED FORK LENGTH (cm.) AT BAND FORMATION
BACK-CALCULATED DATE AND MEAN SIZE AT BAND FORMATION
AUGUST
X̄ = 63 62
N = 15
BAND N° 2
BACK-CALCULATED FORK LENGTH (cm.) AT BAND FORMATION
BACK-CALCULATED DATE AND MEAN SIZE AT BAND FORMATION
OCT. - NOV.
X̄ = 47 37
N = 16
BAND N° 1
BACK-CALCULATED FORK LENGTH (cm) AT BAND FORMATION

Figure 8.—Back-calculated date and mean size at band formation for bluefin tuna (N = 16) of estimated age 2+ from the Bay of Biscay at beginning of season (band numbers 1-3).

Table 1.—Mean back-calculated fork length (cm) at band formation for a 201 cm FL bluefin tuna captured off the Canary Islands in March 1979. Back calculations for this example are given for measurements of the 12 bands or 6 couplets for the dorsal spine cross section. The marginal growth increment (see Glossary) is not included.

	Couplet no. (2 bands per couplet)					
Band no.	1	2	3	4	5	6
1	103.9	121.6	135.0	147.6	171.3	189.9
2	108.3	128.6	140.2	159.7	180.6	198.0

but this assumption could not be validated). The remaining bands corresponded to age 5 to 9 yr, and growth from the last couplet to the margin probably represented growth during the 10th year. These procedures and interpretations were used to assign estimated ages to each specimen in our samples.

Von Bertalanffy Growth Model

Vital parameters for the von Bertalanffy growth curve based on counts of growth bands on spine sections from 221 bluefin tuna indicated an L_∞ = 372.2 cm FL, k = 0.067 (annual), and t_0 = −1.71 yr (Table 2). The growth curve from this study and size at estimated age from Mather (1980) and Farrugio (1979), based on Sella's (1929) data, indicate close (Fig. 10) agreement with our results.

DISCUSSION

Bay of Biscay

The rapid growth of bluefin tuna during their early years of life enables the identification of the first age classes. It is therefore possible to correlate the observed bands (fish collected in the Bay of Biscay) with the ecological conditions to which the fish are subjected. Thus, the first growth band corresponds to

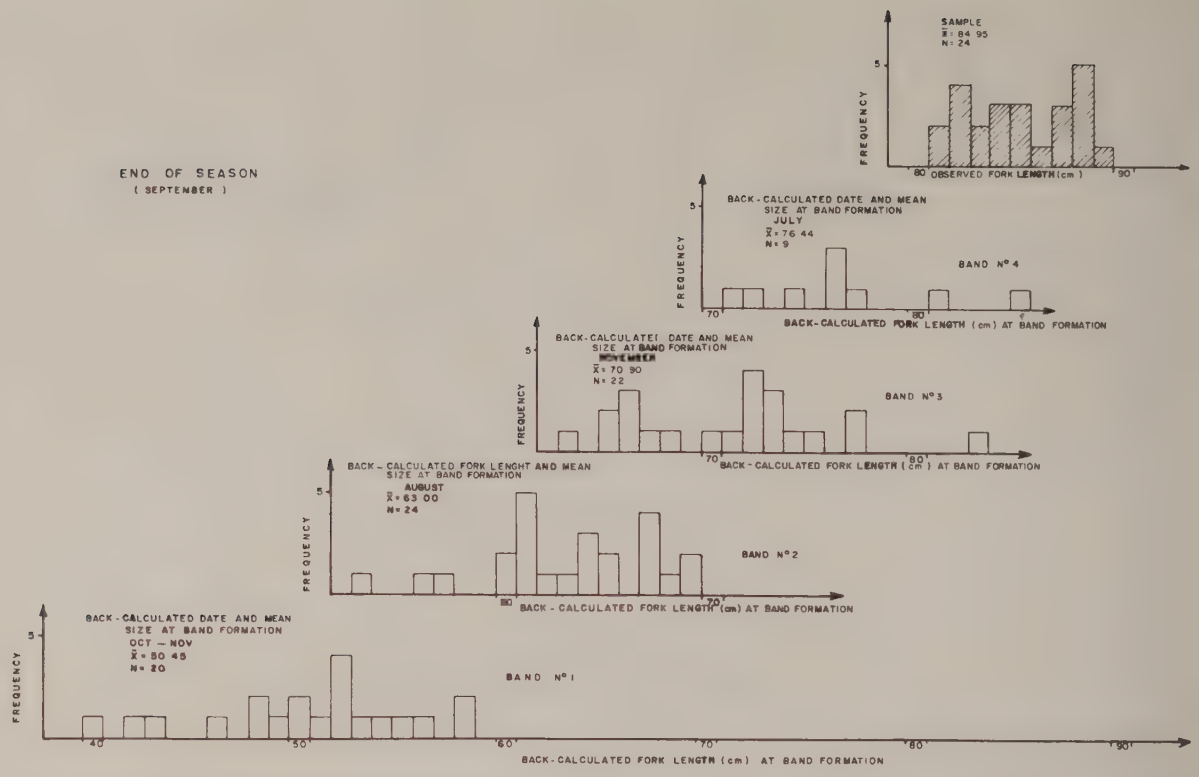
END OF SEASON
(SEPTEMBER)
SAMPLE
X̄ = 84 95
N = 24
FREQUENCY
OBSERVED FORK LENGTH (cm)
BACK-CALCULATED DATE AND MEAN SIZE AT BAND FORMATION
JULY
X̄ = 76 44
N = 9
BAND Nº 4
BACK-CALCULATED FORK LENGTH (cm) AT BAND FORMATION
BACK-CALCULATED DATE AND MEAN SIZE AT BAND FORMATION
X̄ = 70 90
N = 22
BAND Nº 3
BACK-CALCULATED FORK LENGTH AND MEAN SIZE AT BAND FORMATION
AUGUST
X̄ = 63 00
N = 24
BAND Nº 2
BACK-CALCULATED FORK LENGTH (cm) AT BAND FORMATION
BACK-CALCULATED DATE AND MEAN SIZE AT BAND FORMATION
OCT — NOV
X̄ = 50 45
N = 20
BAND Nº 1
BACK-CALCULATED FORK LENGTH (cm) AT BAND FORMATION

Figure 9.—Back-calculated date and mean size at band formation for bluefin tuna (N = 24) of estimated age 2+ from the Bay of Biscay at the end of season (band numbers 1-4).

Table 2.—Vital parameters, mean fork length, and standard error at ages (including sample size) based on measurements of growth bands on spine sections fitted to the von Bertalanffy growth model for 220 bluefin tuna collected from the Canary Islands and Bay of Biscay.

Vital parameters			
	L_∞ = 372.2 cm FL	± 6.13	
	k = 0.0675	± 0.0021	
	t_0 = −1.71 yr	± 0.06	
Estimated ages	Mean fork length (cm)	SE	Sample size
1.0	62.69	0.321	29
2.0	83.05	0.527	41
3.0	102.07	1.711	14
4.0	119.85	1.271	19
5.0	136.47	1.586	19
6.0	152.01	0.832	23
7.0	166.53	0.369	7
8.0	180.10	2.404	3
9.0	192.78	1.098	8
10.0	203.63	1.258	4
11.0	215.71	3.000	2
12.0	226.07	1.455	11
13.0	235.75	0.615	10
14.0	244.80	1.430	6
15.0	253.25	0.667	3
16.0	261.16	0.754	9
17.0	268.54	1.673	5
18.0	275.45	0.957	4
19.0	281.90	1.333	3

the migration from the Mediterranean Sea to the Atlantic coast of Morocco (Sara 1973; Rey 1979). This is supported by the fact that the first band is not present in age class 0 fish caught while they are still in the Mediterranean Sea. In the following two couplets, the first band of each pair (bands 2 and 4, respectively) corresponds to the summer migration from south-

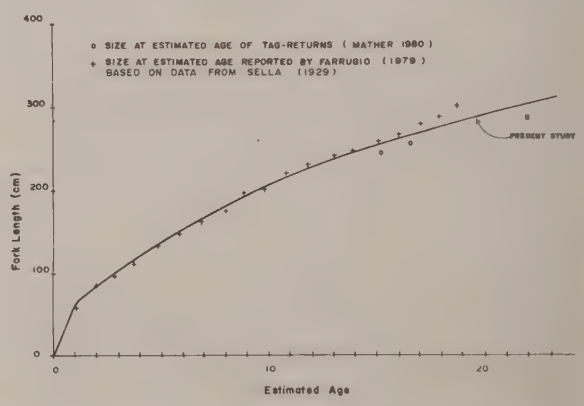
o SIZE AT ESTIMATED AGE OF TAG-RETURNS (MATHER 1980)
+ SIZE AT ESTIMATED AGE REPORTED BY FARRUGIO (1979) BASED ON DATA FROM SELLA (1929)
PRESENT STUDY
Fork Length (cm)
Estimated Age

Figure 10.—Fork length (cm) at estimated age of 227 bluefin tuna caught in the Bay of Biscay, Canary Islands, and Mediterranean Sea, 1978-79, based on counts of bands on dorsal spine cross sections. Size at estimated age (o) based on tag-returns of Mather (1980) and those reported by Farrugio (1979) based on vertebrae (+) from Sella (1929) are also shown.

ern Morocco to the Iberic Coast and the Bay of Biscay. The second band in these same couplets (bands 3 and 5, respectively) corresponds to the tuna's return to the wintering area in the Ibero-Moroccan Bay (Brethes 1979; Lamboeuf 1975). The sixth band or first mark of the third couplet results from a summer migration from Moroccan waters to their point of capture in the Bay of Biscay (Bard 1977).

According to the back-calculated size frequency curves (Figs. 7, 8, and 9), it seems that the band that appears at the end of the fishing season (June to September) in juvenile bluefin tunas is formed just before their arrival in the Bay of Biscay, and it is only visible after their period of growth has started. It is known that juvenile bluefin tuna grow during their stay in the Bay of Biscay. Cort (1976) reported a growth of 15 cm FL in this period for tunas of estimated age 2. In this study, the same growth was found by comparing the mean length of samples at the beginning (Fig. 8) and end of the fishing season (Fig. 9).

Canary Islands

In adult bluefin tuna, the vascularization of bony tissue in the center of the spine causes a loss of early bands. Nevertheless, the sections have bands in the periphery of the spines. These bands show a disposition in pairs and in this work we have considered one couplet for each year. In the majority of sections, up to eight pairs are visible in larger fish (Fig. 11).

The interpretation proposed for the formation of paired bands in adults is as follows: The first band of each annual pair, in the 4th or 5th year, corresponds to the reproductive migration that bluefin undertake from the Atlantic to the Mediterranean Sea during May-June (Rodriguez-Roda 1967). Bluefin tuna larger than 200 cm FL make this migration (Sara 1973). Loss of weight during the spawning migration is substantiated by Rodriguez-Roda (1964). The mean condition coefficient of "right" bluefin tuna (entering the Mediterranean) in June is 2.0, whereas the index of "reverse" fish (leaving) in July-August is 1.6. The bluefin tuna then migrate north, feeding constantly, until they arrive off Norway in August in a well-fattened condition (Tiews 1963). They continue to feed actively during their stay in the North Sea. Within a period of 2 to 3 mo, these fish often attain a 34 to 54% increase of their annual growth in weight (Tiews 1957).

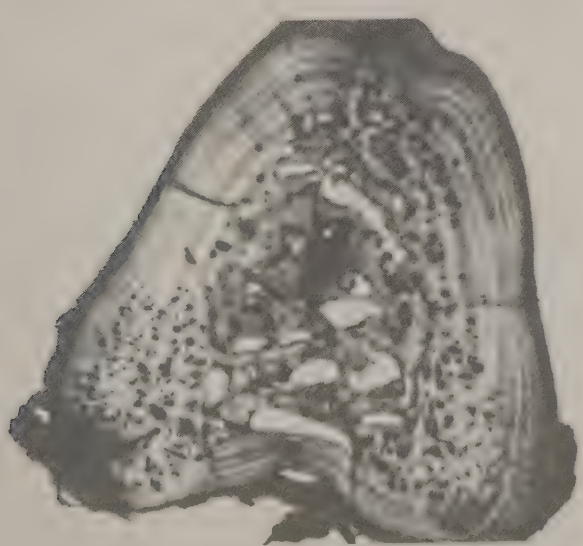

Figure 11.—Section from the first dorsal spine of a bluefin tuna of 201 cm FL, estimated to be age 10+ yr.

The second band is probably formed during their migration to the south in September-October. From November to May, the bluefin tuna remain in the eastern Atlantic between the Bay of Biscay and the Canary Islands. It is well-known that bluefin tuna have less commercial value at the beginning of the fishing season (March-April) because they arrive in a lean condition.

Calculations of the maximum age of bluefin tuna have recently been altered following the recapture of three tagged fish that were at liberty for 13 to 14 yr (Mather 1980). As a result of this work, estimates of longevity have increased from about 21 to 23 yr, to that of at least 30 yr. The recaptured size and estimated age of the fish are plotted in Figure 10.

In the present study, age estimated did not exceed 19 yr. The difference in growth by sex demonstrated by western Atlantic bluefin tuna (Butler et al. 1977) was not examined in this paper.

CONCLUSION

It is interesting to note that migratory movements may be the cause of band formation in bluefin tuna, at least in the eastern Atlantic area. This is particularly true for young, immature fish. For adults, the energetic problems involved in reproduction probably overlap the consequences of migration. Nevertheless, band formation in both young and adult bluefin tunas could be a function of bioenergetic stress associated with migration. This process clearly distinguishes bluefin tuna from other marine, temperate, sedentary fishes in which the reduction or lack of growth in winter reflects physical conditions of the environment. It should be pointed out that Sharp and Dotson (1977) indicated a high probability of lipid utilization as an energy source by migrating albacore, *T. alalunga*. Other temperate tuna that undergo long distance migrations probably register two growth bands each year (Bard and Compeán-Jimenez 1980).

The use of spinal sections to estimate age has the advantage of easy sampling and the growth bands stand out clearly. An additional advantage of the method is easy storage of samples for future reexamination. However, estimates of age from spine cross sections has not been validated and further research is necessary to identify migrations as a probable, primary cause of growth band formation.

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SUMMARY PAPER

Deterministic Partitioning of the Catch of Southern Bluefin Tuna, *Thunnus maccoyii*, into Age Classes Using an Age-Length Relationship[1]

JACEK MAJKOWSKI and JOHN HAMPTON[2]

INTRODUCTION

Southern bluefin tuna, *Thunnus maccoyii*, are a highly migratory species intensively exploited by Australian and Japanese fishermen at various stages of the fish's life cycle. The biology and fisheries of this species are reviewed by Hynd (1965), Murphy (1977), Shingu (1978), Majkowski, Williams, and Murphy (1981), and Murphy and Majkowski (1981). Catch information from both fisheries constitutes the main input data for many of the routine analyses for stock assessment. Most of these analyses require not only gross annual catch values, but also estimates of these catches partitioned into age classes (see above references).

In this paper we present a deterministic method of partitioning the annual catch of southern bluefin tuna into age classes using an age-length relationship. Potential sources of errors in the partitioned catch estimates are outlined, and the errors in these estimates, attributable to known uncertainties in parameters of the age-length relationship, are quantitatively assessed with the aid of Monte-Carlo simulations. Knowledge of these errors and the requirements of the assessment procedures for which the catch estimates are being used as input data are essential if the degree of confidence of population assessment is to be determined (Majkowski 1982, in press).

METHODS

Input Data for the Partitioning Procedure

Estimates of the catches by length class and fishing period constitute the input data for the partitioning procedure. These catch arrays were constructed on the basis of gross catch data and information from routine length-frequency sampling in Australian canneries and on board Japanese longline vessels (Williams 1982a, b). One and 2 cm length classes were used in the Australian and Japanese catch sampling programs, respectively. The sampling information was grouped by half-month (Australian fishery) and quarter-year (Japanese fishery) periods (Hampton 1982a, b) and used in conjunction with the gross catch data for these periods.

Age-Length Relationship

Kirkwood[3] has derived the parameter values ($t_0 = -0.429$ yr, $k = 0.127$, $L_\infty = 207.7$ cm) of the following von Bertalanffy growth equation for southern bluefin tuna from mark recapture and length mode data:

$$t = t_0 - \frac{1}{k}\ln(1 - L/L_\infty) \quad (1)$$

where t denotes the estimated age (in years) and L denotes the fork length (in centimeters). This relationship was used as the basis for partitioning the catch values into age classes.

Partitioning Procedure

Fish lengths from any length class were assumed to be uniformly distributed within the length range of that length class. For each array element (i.e., the catch by length class and fishing period), the following computations were performed: 1) Estimated age, t, was calculated on the basis of Equation (1) using, in turn, the lower and upper boundaries of the length class. 2) Dates of birth corresponding to both values of t were calculated by subtracting t from the date of capture (assumed to be the midpoint of the period for which the length frequency was constructed). Fish having a birth date between 1 July of year X and 30 June of year $X + 1$ were assigned to a cohort denoted by $X + 1$. This assignment is consistent with our knowledge of the spawning period (September-March) of the species (Shingu 1978). If the assigned cohorts relating to both the upper and lower length boundaries were the same, all fish from that length class were assumed to belong to the one cohort. However, if the two assigned cohorts were different, a cohort boundary existed within the length class. The catch number was then apportioned between the two cohorts according to the exact position of the cohort boundary within the length class. 3) The age class was identified by the number defined as being one more than the difference between the year of capture and the year denoting cohort, e.g., estimated age class 2 refers to fish estimated to be between 1 and 2 yr old. The total year's catch processed in this way resulted in a series of catch estimates by age class (C_i's).

[1]Some information contained in this paper was summarized from a manuscript submitted to the Canadian Journal of Fisheries and Aquatic Sciences.

[2]Commonwealth Scientific and Industrial Research Organization (CSIRO), CSIRO Marine Laboratories, Division of Fisheries Research, P.O. Box 21, Cronulla, New South Wales 2230 Australia.

[3]Kirkwood, G. P., Senior Research Scientist, Commonwealth Scientific and Industrial Research Organization, CSIRO Marine Laboratories, Division of Fisheries Research, Cronulla, New South Wales, Australia. Unpubl. data.

Sources of Uncertainties

The errors in C_i's are contributed to by two uncertainties: 1) The von Bertalanffy growth equation (i.e., in the form of this equation and its parameter values), and 2) the length frequency data (i.e., in the sampling and gross catch information). Since only information on the uncertainties in t_0, k, and L_∞ exists, only the effect of these uncertainties on the estimation of accuracy of C_i's can be assessed.

Method of Accuracy Analysis

Kirkwood (footnote 3) has estimated the variance-covariance matrix for the southern bluefin tuna growth parameters:

	t_0	k	L_∞
t_0	0.004	—	—
k	0.0002	0.00002	—
L_∞	−0.12	−0.011	8.4

The multivariate normal distribution defined by the mean values of t_0, k, and L_∞ and the associated variance-covariance matrix were assumed to represent the growth parameter uncertainties. Values of t_0, k, and L_∞ were stochastically sampled from this distribution using an IMSL[4] computer subroutine. Monte-Carlo simulations (Miller 1974; Miller et al. 1976; Garten et al. 1978; O'Neill and Gardner 1979; Gardner et al. 1980; O'Neill et al. 1980; Majkowski, Ridgeway, and Miller 1981; Majkowski 1982, in press; Powers 1983) of C_i's were performed using these parameter values in the catch partitioning procedure. Because the simulated values of L_∞ never fell below the largest observed fish length, all lengths could be classified into age classes for all simulations. We assumed that the distributions of C_i values reflected their uncertainties.

RESULTS

Results of partitioning the 1970 catch into estimated age classes and cohorts are presented, as an example, in Table 1. It is evident from these results that the catches of fish younger than 1 yr old and older than 17 yr old are extremely small.

[4]International Mathematical and Statistical Libraries, Inc., 7500 Bellair Boulevard, Houston, TX 77036. Routine GGNSM (stochastic sampling from a multivariate normal distribution). Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1.—The 1970 global catch of southern bluefin tuna, *Thunnus maccoyii*, classified into estimated age classes and cohorts.

Estimated age class	Assumed cohort	Estimated catch (no. of fish)
1	1970	690
2	1969	68,312
3	1968	695,798
4	1967	163,115
5	1966	88,702
6	1965	104,458
7	1964	53,714
8	1963	68,485
9	1962	85,441
10	1961	111,059
11	1960	89,625
12	1959	60,941
13	1958	35,534
14	1957	15,453
15	1956	8,137
16	1955	3,271
17	1954	1,173
18	1953	480
19	1952	274
20	1951	191

The percentage deviation (D_i) of the simulated C_i values from those C_i values obtained using the best estimates of t_0, k, and L_∞ is presented for the 1970 length-frequency data (Fig. 1). The graphical presentation was prepared on the basis of 500 Monte-Carlo simulations, although a much smaller number of simulations provided nearly identical information on the statistical distribution of D_i values. Two major observations emerge from an examination of Figure 1: 1) The mean values of D_i's are relatively close to zero only for age classes 3 to 13, and 2) the ranges of D_i values bounded by the 2.5 and 97.5 percentiles (i.e., encompassing 95% of the D_i values) are relatively narrow only for the above-mentioned age classes. The D_i values for age classes 3 to 13 were approximately normally distributed. The standard errors of D_i's (the coefficients of variation of C_i's) for these age classes were $< 12\%$.

DISCUSSION

The major advantage of the partitioning method is its ease of use. Once a growth curve has been determined (e.g., on the basis of mark-recapture studies, mode progression analysis, and/or the analysis of growth rings on hardparts), the classifi-

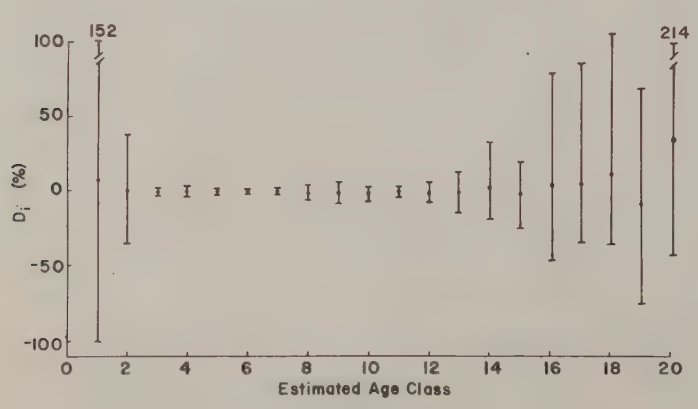

Figure 1.—The mean values (dots) and ranges bounded by the 2.5 and 97.5 percentiles (solid bars) of D_i, both obtained on the basis of 500 Monte-Carlo simulations.

cation of the catch into age classes is very simple if the information on length-frequencies of the catch is available.

Only errors in the age-structured catch estimates relating to the growth parameter uncertainties have been addressed in this paper. From this analysis it follows that the estimates for age classes 1, 2, and 14 to 20 are very unreliable and should not be used as input information for southern bluefin tuna stock assessments.

The relationship between C_i uncertainty and age class is determined by the probability distributions of t_0, k, and L_∞, the form of the age-length relationship, and the fish-length frequencies. The relatively high uncertainties in the estimated catches of age classes 14 to 20 may be due to: 1) A reduction in the growth rate with increasing age, 2) an increase in the overlap in the length distributions of these age classes, and 3) the length-dependent predictive power of the age-length relationship (the uncertainty in the predictions of ages from lengths increases towards the extremes of the length range). The bias in catch estimates derived in a similar way to that presented by us, which result from the overlapping length distributions of older age classes, is considered by Bartoo and Parker (1983). In addition to cause 3), the high uncertainties associated with age classes 1 and 2 can be ascribed to the size selectivity of the fishing method used and the relatively small catches of these fish (Table 1). As a result of these, the length distributions of young fish caught may be different from those of fish in the entire population. In addition, the length frequencies of the two youngest age classes may be subject to large sampling error, this being a consequence of the small catches (catches are sampled randomly). While most simulations result in only a small absolute number of young fish being reclassified, say, from age class 3 to age class 2, the relative change in numbers might be very large for age class 2 because of its small estimated catch number. Similar factors may also affect the catch estimates for age classes 14 to 20.

The variance-covariance estimates of t_0, k, and L_∞ are contributed to by errors in the data set used for the estimation of these parameters. The unsuitability of the form of age-length relationship and variabilities in the growth rate from fish to fish, year to year, and even area to area, are also reflected in the variance-covariance estimates. Hence, we can presume that error due to improperly accounting for these variabilities in the partitioning procedure is not large for catch estimates of age classes 3 to 13. This conclusion can be verified when a direct ageing method (i.e., reading hardpart sections) is developed for southern bluefin tuna, allowing the precise determination of these variabilities.

The effect of measurement errors on the values of t_0, k, and L_∞ seems small because the mark-recapture and length-mode data sets used in their estimation are very large and give no indication of bias. Some reductions in the k and L_∞ uncertainties may be possible if more recaptures of very old fish are added to the data set. The growth curve used for ageing southern bluefin tuna does not account for seasonal changes in the growth rate but this changeability, if identified, can be easily incorporated into the ageing process by replacing Equation (1) with a more complex formula (such as those presented by Pitcher and MacDonald 1973; Pauly 1982).

It may be possible to properly account in the partitioning method for the age-dependent growth variability among fish (for examples of such methods, see Schnute and Fournier 1980; Clark 1981; Bartoo and Parker 1983) if some information on this variability is derived. The extent of information required for the application of each of these methods is different.

If the southern bluefin tuna growth rate for all ages is significantly variable from year to year, area to area, or both, the only possibility of decreasing the uncertainty in the partitioned catch estimates attributable to this variability is to use the unbiased age-length key method (Westrheim and Ricker 1978). This method could be applied if an efficient method of directly ageing southern bluefin tuna is fully developed. Possibly the catches of fish younger than 4 or 5 yr old could also be reliably aged in this case on the basis of a distribution mixture method (MacDonald and Pitcher 1979; McNew and Summerfelt 1978), because modes in the length frequencies for these ages are easily distinguishable.

Uncertainties in the C_i estimates are caused not only by the weaknesses of the partitioning procedure but also by errors in the input data for the procedure (i.e., catch estimates by length class and fishing period). These errors may also contribute significantly to the C_i uncertainties. Therefore, more attention should be paid to the design of a system of collecting the data and to uncertainties in the information being derived from the system.

Until a method of ageing southern bluefin tuna based on hardpart analysis is developed, the use of the growth curve to partition catches into age classes is our only ageing option. The present study has quantified the uncertainties in C_i estimates, indicating that the catch estimates for estimated age classes 1, 2, and 14 to 20 cannot be used for population analyses.

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SUMMARY PAPER

Progress of Age and Growth Assessment of Atlantic Skipjack Tuna, *Euthynnus pelamis*, from Dorsal Fin Spines[1]

LOIC M. ANTOINE,[2] JEREMY J. MENDOZA,[2] and PATRICE M. CAYRÉ[3]

ABSTRACT

The present study is a part of an ongoing international research program on Atlantic skipjack tuna, *Euthynnus pelamis*, coordinated by the International Commission for the Conservation of Atlantic Tunas (ICCAT). Methodology was developed for estimating age and growth rate based on counts of growth bands on sections of dorsal fin spines from 78 skipjack tuna.

The precision of counts of growth bands between eight different readers is assessed and the difficulties encountered in developing methodology and differences between readers were identified. A preliminary estimate of growth rate is presented based on samples from three origins. Estimates of age based on counts of growth bands on spines remain unvalidated, particularly the assumption of two bands per year we used for interpretation. However, ongoing studies using tetracycline as an internal tag to determine the periodicity of growth marks indicate this substance is deposited on spines, but longer times at liberty (> 1 yr) will be necessary for more definitive results.

RÉSUMÉ

Cette étude fait partie d'un programme international de recherche sur le listao de l'Atlantique, *Euthynnus pelamis*, coordonné par la Commission Internationale pour la Conservation des Thonidés de l'Atlantique (CICTA). Une méthodologie est proposée pour estimer l'âge et le taux de croissance; elle est fondée sur l'étude des bandes de croissance lues sur des coupes de rayons de la nageoire dorsale chez 78 individus.

La précision relative de lecture a été étudiée chez huit expérimentateurs; les difficultes pour mettre au point cette méthodologie, ainsi que les différences entre expérimentateurs ont été abordées. Une estimation préliminaire du taux de croissance sur trois échantillons d'origines différentes est présentée. L'estimation de l'âge à partir des bandes de croissance sur les coupes d'épines nécessitent une validation, et particulièrement l'hypothèse faite sur la formation de deux bandes par an. Cependant les études en cours au moyen de tétracycline comme marqueur interne montrent que cette substance est déposée dans l'os des épines et peut aider à déterminer la périodicité des marques de croissance, mais il faudrait des temps de liberté plus longs (un an ou plus) que ceux observés à présent pour obtenir des résultats consistants.

INTRODUCTION

Different approaches have been taken for estimating the age and growth rate of skipjack tuna, *Euthynnus pelamis*. A synopsis of past work is presented by Josse et al. (1979) and includes a review of length frequency analysis, modal progressions, mark and recapture studies, and counting growth bands on hardparts (i.e., vertebrae, otoliths, and dorsal fin spines). The rates of growth reported by different authors were quite variable and in some cases differences between studies were as much as two- or three-fold. These differences may be partially attributed to the diversity of methods and origins of samples.

The International Commission for the Conservation of Atlantic Tunas (ICCAT) is responsible for making management recommendations for Atlantic scombrids and implemented the International Skipjack Year Program (ISYP) in 1981. Part of this research effort, with emphasis on skipjack tuna recommended by the ICCAT working group, included age and growth rate assessment of skipjack tuna in the eastern Atlantic Ocean. The objectives of this study were to develop a technique for estimating age and growth rate of skipjack tuna based on counts of growth bands on spine sections and to assess the precision of these counts by different readers. We chose the dorsal fin spine as a source of age and growth information because of the ease and utility of this structure reported by Shabotinets (1968), Batts (1972), and Cayré (1979) for estimating age and growth rate of skipjack tuna.

METHODS AND MATERIALS

Our approach to age and growth assessment of skipjack tuna was developed during a series of meetings of the ICCAT skipjack working group (four scientists) held in Brest, France, and Dakar, Senegal, during 1980 and 1981. Specimens used for this

[1]This research is part of the International Skipjack Year program coordinated by the International Commission for the Conservation of Atlantic Tunas.

[2]Centre Oceanologique de Bretagne, B.P. 337-29273, Brest Cedex, France.

[3]Centre de Recherche Oceanographique, B.P. 2241, Dakar, Senegal.

analysis were obtained by sampling purse seine and bait-boat landings during 1980 in Senegal, Ivory Coast, and Venezuela.

The first dorsal spine was extracted from each specimen and the fork length (cm FL), total weight (g), date of capture, and location were recorded. A series of three sections (500-700 μm thick) were cut from the spines above the condyle base (3-5 mm according to length of individual fish), using an Isomet[4] low-speed saw.

Spine sections were mounted in a drop of 90% alcohol and viewed under a projector with transmitted light or with a binocular lens microscope using incidental light and a dark background. Sections were roughly cone-shaped and examinations were restricted to the distal surface of each section (side farthest to the condyle base). Sections of the second dorsal fin spine were also examined (when available) to aid interpretation. Translucent growth zones (see Glossary) appeared clear in transmitted light and dark in incidental light, whereas opaque growth zones were dark in transmitted light and light in incidental light. X-ray microradiographs done on several spine sections indicated that translucent bands represented zones of higher calcium concentration, which have been reported to represent areas of inhibited (slow) growth (Castanet et al. 1977; Compeán-Jimenez and Bard 1980). A series of photographs of spine sections were compiled and distributed to eight readers for counting, measuring, and interpreting growth bands.

We use the term "ring" to refer to translucent zones which were counted on each specimen. A code was defined to enable readers to standardize their interpretations. Previous reports indicate that rings on spines of Pacific and Atlantic tunas are often present in groups of two or more, which may represent annual cycles (Chi and Yang 1973; Compeán-Jimenez and Bard 1980; Cayré and Diouf 1981). Our observations also suggest this hypothesis for skipjack tuna and thus we have adapted this assumption for interpreting groups of rings to estimate age. Therefore, each group of rings we identified was assumed to represent 1 yr of growth. Owing to the sparse knowledge of the biology, life history, and behavior of skipjack tuna in different geographical areas, it was not possible to recognize rings as "accidental," "spawning checks," or attributable to other biological or environmental events.

Our code for rings, used by seven out of eight readers (reader 4 was unaware of the existence of this code), was as follows:

A = ring
AR = ring present in vascularized core
AF = blurry ring; not well marked; limits slightly marked
AE = narrow ring
AL = large ring
Ai = incomplete ring
Ad = ring partially split along the longitudinal axis
A^{l} = ring particularly well marked.

The reader described each section by this code and then indicated the ring counts or groups that he used to assign an age to each sample. An example of our interpretation follows:

$$\frac{\text{Code and ring number}}{\text{Estimated age}} \quad \frac{AR}{1} + \frac{AE + AL}{2} + \frac{A^{l} + AF}{3} + \frac{A + A}{4} + \frac{A}{+}$$

This example represents a total of eight rings with an estimated age of 4+ yr.

Measurements were taken with a profile projector fit with a stage coupled with a micrometer and a binocular lens microscope fit with an ocular micrometer. Measurements taken on spine cross sections included: 1) Spine diameter (d)—the distance between the outside margins of the spine above the notch in the posterior face through the approximate center of the spine (Fig. 1), 2) radius of growth band (r)—the distance from the estimated center of the spine to the outside margin of each growth increment, and 3) diameter of growth band (d^{1})—the distance from the outside spine margin through the spine center to the outside margin of each growth band (Fig. 1).

When using a profile projector, a line was drawn through the center axis (a^{1} to a^{2}), bisecting the spine in the mid-sagittal plane (Fig. 1). The location where this line (a^{1} to a^{2}) intersects the spine diameter (d) was the estimated center of the spine.

Depending on the measurement, the radius of each growth band was given by the value of r or ($d^{1} - d/2$). A t-test of the mean values of the difference between r and ($d^{1} - d/2$) for 30

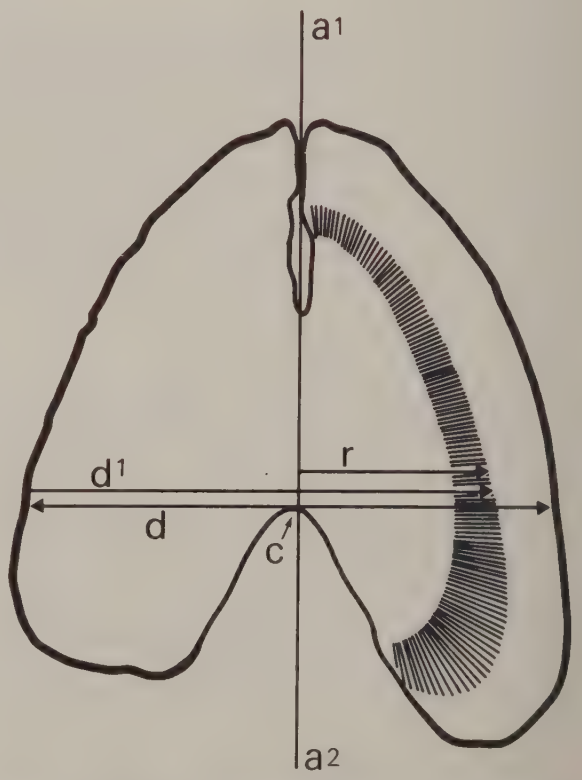

Figure 1.—Cross section of dorsal fin spine of skipjack tuna. a^{1}-a^{2} = sagittal plane; c = estimated center of the spine; d = spine diameter; d^{1} = growth ring diameter; r = growth ring radius.

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

different section readings did not show a significant difference ($\alpha = 0.05$) between the two methods; therefore, observations from both were pooled for ageing analysis.

In skipjack tuna ≥ 50 cm FL, the first several rings were often obscured (masked) due to enlargement of the vascular core. We attempted to resolve the problem following the general methods outlined by Cayré and Diouf (1983), Berkeley and Houde (1983) and Gonzales-Garces and Fariña-Perez (1983). This approach entails calculating the average number and location of the first several bands observed in very young fish to correct for obscured bands in larger (older) individuals.

In order to compare interpretations of different readers, photographs of 78 dorsal spine sections were sent to eight readers. The readers did not have the characteristics of the fish (length, origin), in order to avoid biasing the readings. The photographic magnification of all prints was the same. Readers 1, 2, 3, 5, and 7 participated in developing the reading code and applied it, readers 6 and 8 applied the code without having participated in its development, and reader 4 did not apply the method code for age estimation but rather counted his interpretation of annual bands to assign an age. The 78 samples were deliberately chosen from fishes coming from different origins (Caribbean, central Atlantic, Gulf of Guinea), and for this reason we will not try to interpret results from the point of view of skipjack tuna growth since the major objective of this experiment was to determine the level of agreement between readers.

A mean age was initially calculated for the spine sections read by each reader. Variances between readers were tested for homogeneity and were found to be significantly different (F max test; $\alpha \leq 0.05$). Therefore, statistical comparisons between readers was accomplished by establishing an age-length relationship for each reader's data set. We chose to represent length as a function of age by a least squares linear model and this yielded predictive regression lines for each reader (an example is given in Fig. 2). Because residual variances of the different regressions were not homogenous, variance analysis was not used to compare the regression lines. An alternative approach using the joint confidence region for a given probability level for both slope and elevation of the regression lines was adopted (Draper and Smith 1966). This region takes the shape of an elongated ellipse. Differences between paired estimates for elevation and slope between readers were declared significant when the ellipses did not intersect. Details of the method are given by Conan (1978). All statistical inferences were made with a significance level of $\alpha = 0.05$.

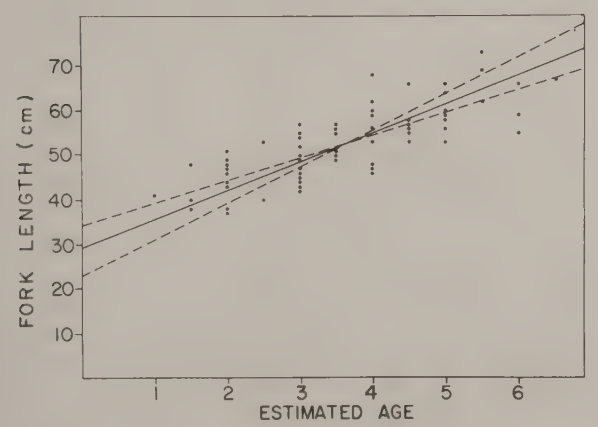

Figure 2.—Example of fork length vs. estimated age regression obtained for reader 5. Solid line is the functional regression, dashed lines are the predictive regressions.

Two different methods (back calculation and age-length relationships) were used to study growth. The estimated length at different ring formation based on spine measurements was determined by back calculation. This method increases the number of observations but may be biased from the dependence of the different age-length estimates and from the correction of obscured rings in larger fish.

For growth estimated by back calculation, the predictive regressions obtained for each sample were used in calculations. The formula used in back calculations follows Lee (1920):

$$FL_i = a + (FL - a)\frac{A_i}{A} \quad (1)$$

where FL_i = fork length at time i
FL = observed fork length
a = bias adjustment parameter
A_i = radius of ring
A = radius of section.

We also examined growth by observing estimated age-length relationships; this method tends to lend itself better for adjustment to mathematical models.

RESULTS AND DISCUSSION

From a total of 78 photographs of spine sections, 17 (21.8%) were considered unreadable by at least one person. Only one specimen (1.3%) produced total agreement among all readers, and two others produced agreement when interpreted to within ± 0.5 yr (assuming two rings per year, 0.5 yr is represented by one isolated ring). This represents a total agreement within ± 0.5 yr of 3.8%. It is noteworthy to mention that a similar comparison on cod otolith readings showed 39% agreement between 10 readers (Lopez-Veiga et al. 1977). Berkeley and Houde (1983) found that only 13% of swordfish, *Xiphias gladius*, spines were unreadable. Therefore, it appears that agreement between readers was unusually low in our study, and unreadable spines are relatively numerous compared with what we had expected and as indicated in other reports.

Comparisons between different pairs of readers (Table 1) indicated < 40% agreement, except for readers 2 and 4 (56%) and readers 7 and 8 (73%). Lowest values were between read-

Table 1.—Percent agreement between pairs of readers for counts of rings on cross sections of 78 skipjack tuna spines captured off Venezuela, Senegal, and Ivory Coast, 1980-81.

	Agreement between pairs of readers (%)							
Reader	1	2	3	4	5	6	7	8
1		31	38	31	30	23	13	14
2	31		25	56	31	9	13	10
3	38	25		20	39	24	38	30
4	31	56	20		24	8	16	13
5	30	31	39	24		14	23	26
6	23	9	24	8	14		21	21
7	13	13	38	16	23	21		73
8	14	10	30	13	26	21	73	

ers 4 and 6 (8%) and 2 and 6 (9%). Close agreement between readers 7 and 8 could be related to their close geographical proximity, which gave them an opportunity to work together longer during the development of the methodology in their laboratory. In addition, these readers did not attempt to age within ± 0.5 yr. However, readers 2 and 4 also achieved a comparatively high level of agreement even though they used different methods and did not work together.

When comparisons between pairs of readers were tabulated for counts to within ± 0.5 yr, a much higher rate of agreement was observed (Table 2). Sixteen pairs of readers had an agreement rate that exceeded 50% and 13 pairs of readers exceeded 60% agreement. The low rates of agreement may be related to reading closely spaced rings near the outer margin of the sections. This has also been shown to be a problem in reading bluefin tuna, *Thunnus thynnus*, vertebrae (Lee et al. 1983).

The mean bias shown in Tables 3 and 4 is a comparative index defined as the sum of overestimated and underestimated age and illustrates the tendency of a reader to count rings in relation to the entire set of readings. Thus, readers 2 and 4 clearly tend to underestimate age compared with the other readers (indicated by a minus sign), while readers 7 and 8 clearly overestimate age. Readers 3, 5, and 6 slightly overestimated age and reader 1 slightly underestimated age.

The coded interpretation from each reader indicated that, except in several particularly easy cases with well-marked rings, there was considerable variation in the counts and measurements of rings by individual readers. We felt these discrepancies were due, in part, to differences in the individual reader's ability to recognize groups of rings.

Figure 3 shows that two groups of readers may be clearly distinguished by non-overlapping ellipses (i.e., these groups were significantly different from each other): 1) readers 3, 5, 6, 7, and 8, 2) readers 2 and 4. Reader 1 occupies an intermediate position between these groups. Readers 3, 5, and 6, and readers 7 and 8 may also be grouped (quasi-concentrical ellipses). Parameters of the functional and predictive regressions of these analyses are given in Table 5.

The determination of age in skipjack tuna by the use of dorsal fin spines remains difficult. Even when a common methodology is used, interpretations show important divergences.

Table 2.—Percent agreement between pairs of readers within ± 1 ring for counts on cross sections of 78 skipjack tuna spines from Venezuela, Senegal, and Ivory Coast, 1980-81.

	Agreement between pairs of readers (%)							
Reader	1	2	3	4	5	6	7	8
1		67	66	62	61	50	40	46
2	67		48	72	41	54	25	22
3	66	48		46	63	65	67	61
4	62	72	46		35	50	26	24
5	61	41	63	35		60	45	48
6	50	54	65	50	60		66	61
7	40	25	67	26	45	66		73
8	46	22	61	24	48	61	73	

Table 3.—Bias between percent pairs of readers within ± 0.5 yr. Bias is measured as % overestimated average age, − % underestimated average age. These values are only relative in the comparative sense since absolute age is not known.

	Agreement between pairs of readers (%)								
Reader	1	2	3	4	5	6	7	8	Mean bias
1		49	−26	47	−24	−23	−42	−64	−12
2	−49		−68	5	−61	−63	−64	−90	−56
3	26	68		70	−2	2	−42	−46	11
4	−47	−5	−70		−57	−41	−42	−87	−50
5	24	61	2	57		5	−29	−27	14
6	23	63	−2	41	−5		−45	−42	5
7	42	64	42	42	29	45		1	38
8	64	90	46	87	27	42	−1		50

Table 4.—Bias between percent pairs of readers within ± 1 yr. Bias is measured as % overestimated average age, − % underestimated average age. These values are only relative in the comparative sense since absolute age is not known.

	Agreement between pairs of readers (%)								
Reader	1	2	3	4	5	6	7	8	Mean bias
1		31	−20	36	−23	−16	−54	−44	−13
2	−31		−51	9	−57	−44	−76	−79	−47
3	20	51		54	0	−13	−27	−29	8
4	−36	−9	−54		−58	−50	−74	−76	−51
5	23	57	0	58		−3	−31	−29	11
6	16	44	13	50	3		−22	−28	11
7	54	76	27	74	31	22		1	41
8	44	79	29	76	29	28	−1		41

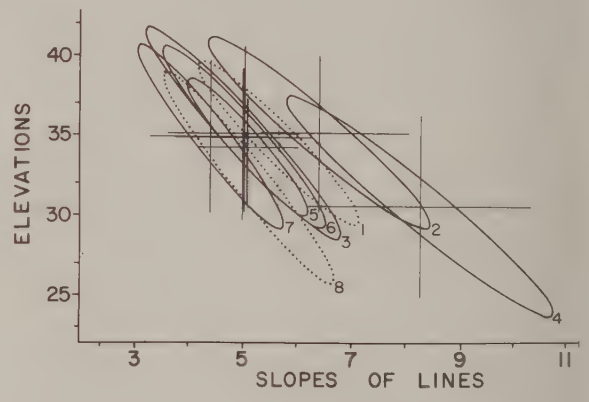

Figure 3.—Ellipses of joint confidence limits for slope and evaluation for the relationship between fork length and estimated age (see details in text) for 8 readers. Readers grouped together are: 3, 5, 6, 7 and 8; 2 and 4. Reader 1 is transitional between the groups. Solid and dotted vertical and horizontal axis for each ellipse denote the elevation (y intercept) and slope, respectively.

Table 5.—Parameters of functional and predictive regressions FL = $a + b$ (age) for each reader where a = intercept, b = slope, r = coefficient of correlation, N = number of individuals.

	Functional regression		Predictive regression			
Reader	a	b	a	b	r	N
1	27.57	7.77	34.51	5.65	0.727	78
2	25.72	9.78	35.14	6.40	0.654	77
3	24.06	8.12	35.12	5.01	0.616	75
4	21.98	1.47	30.55	8.27	0.721	61
5	29.23	6.47	34.25	5.09	0.787	76
6	26.34	7.39	34.86	5.03	0.680	78
7	26.14	6.54	34.90	4.41	0.674	78
8	22.19	7.60	32.32	5.10	0.671	78

Differences arise from the number of rings seen and coded and from the way in which these are grouped. The absence and/or the blurry nature of rings in the altered central zone most likely increases the bias in readings, especially when the fish are more than 50 cm FL. Finally, the nature of the edge of the sections is difficult to interpret. Nevertheless, the use of a common methodology allows comparisons of precision between readers. When possible, samples should be read by several investigators before drawing any conclusion on skipjack tuna age and growth. Although we considered the precision of our age estimates, accuracy of these estimates (see Glossary) was not addressed.

Our results show that there was a comparatively high level of agreement between readers 1, 3, and 5 (Tables 1-5). Each of these readers examined samples from landings at Cumana, Venezuela (N = 150), from Dakar, Senegal (N = 49), and from Abidjan, Ivory Coast (N = 99), and regression lines were adjusted to estimates of age at length (Fig. 4). The comparison between regression lines from the three areas was done by means of ellipses of joint confidence limits because the residual variances between areas were not homogenous (F max test significant; α = 0.05). Figure 4 indicates that samples from these three areas could not be statistically distinguished from each other.

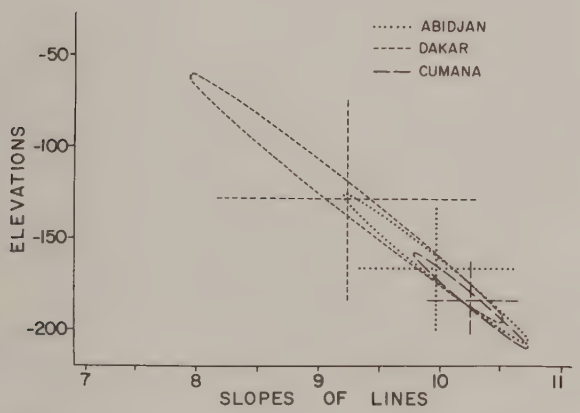

Figure 4.—Ellipses of joint confidence limits for slope and elevation of ring radius vs. fork length regressions for three samples from Ivory Coast (dotted line), Senegal (small dashed line), and Venezuela (large dashed line). Vertical and horizontal axes for each ellipse denote the elevation (y intercept) and slope, respectively.

We found that rings within the central altered zone of sections, especially for fish with fork lengths > 50 cm, were often obscured. The measurements of growth rings (Fig. 5) from each fish identifies (on the average) the location of the first three rings (800, 1,000, and 1,300 μm, respectively). These data were used to estimate rings obscured in fish larger than 50 cm FL due to enlargement of the core.

The significant relationship between the diameter of the dorsal fin spine section and fork length (Table 6) provides strong rationale for back calculation of length at ring formation. The fork lengths at estimated age based on back calculation and from observed data (Table 7) indicate about 4 to 5 cm FL between cohorts. There was a significant relationship between estimated age and fork length for each of the three areas (Fig.

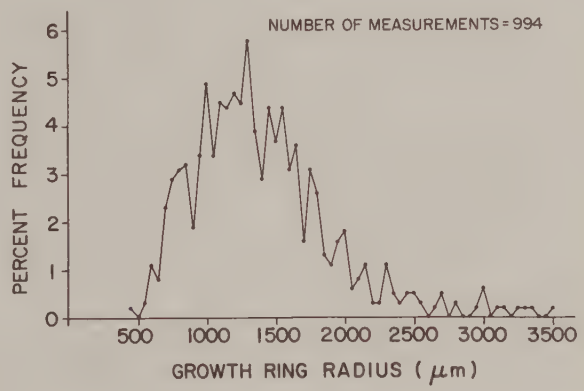

Figure 5.—Frequency of growth ring radius for 994 measurements in all skipjack tuna spine samples combined.

Table 6.—Parameters for regression analysis of the relationship between fork length and spine diameter by location. FL = a + bd where: a = elevation; b = slope; r = coefficient of correlation; N = sample size.

Location	a	b	r	N
Cumana (Venezuela)	19.6722	0.09275	0.88	150
Abidjan (Ivory Coast)	19.8645	0.09133	0.84	99
Dakar (Senegal)	19.4613	0.09216	0.88	49

Figure 7.—Fork length (cm) at estimated age obtained by back calculation and average fork length at estimated age based on spine analysis from three geographical areas. FL = fork length; SD = standard deviation.

Estimated age	Cumana (Venezuela)				Abidjan (Ivory Coast)				Dakar (Senegal)			
	Back calculation		Average fork length at estimated age		Back calculation		Average fork length at estimated age		Back calculation		Average fork length at estimated age	
	FL	SD	FL	SD	FL	SD	FL	SD	FL	SD	FL	SD
1	34.10	1.81	34.68	4.60	34.50	2.21	35.75	4.89	34.20	12.00	35.24	3.69
2	39.00	2.70	39.09	4.57	38 80	2.72	39.92	4.84	39.50	2.55	40.27	3.47
3	44.10	2.90	43.50	4.54	43.20	3.08	44.09	4.80	45.10	2.87	45.30	3.49
4	47.90	2.95	47.91	4.53	47.50	3.52	48.26	4.78	49.80	2.08	50.33	3.46
5	51.60	3.69	53.32	4.52	52.40	4.68	52.43	4.77	54.00	3.16	55.36	3.47
6	53.60	5.13	56.73	4.52	55.60	5.54	56.60	4.78	57.70	3.69	60.39	3.53
7	62.80	6.18	61.14	4.53	58.70	3.78	60.77	4.81				

6), but more detailed analyses were not justified, because the first few rings obscured by the vascularized core were all corrected from the same pooled data base (Fig. 5). Although the observed and back-calculated fork length and estimated ages were very close (Table 7), we did find slightly higher values from Dakar. Statistical comparisons of these data were not made because of the heterogeneity of sample variances. Overall, these data tend to verify that skipjack tuna from the three geographical areas were generally reacting to the same environmental stimuli.

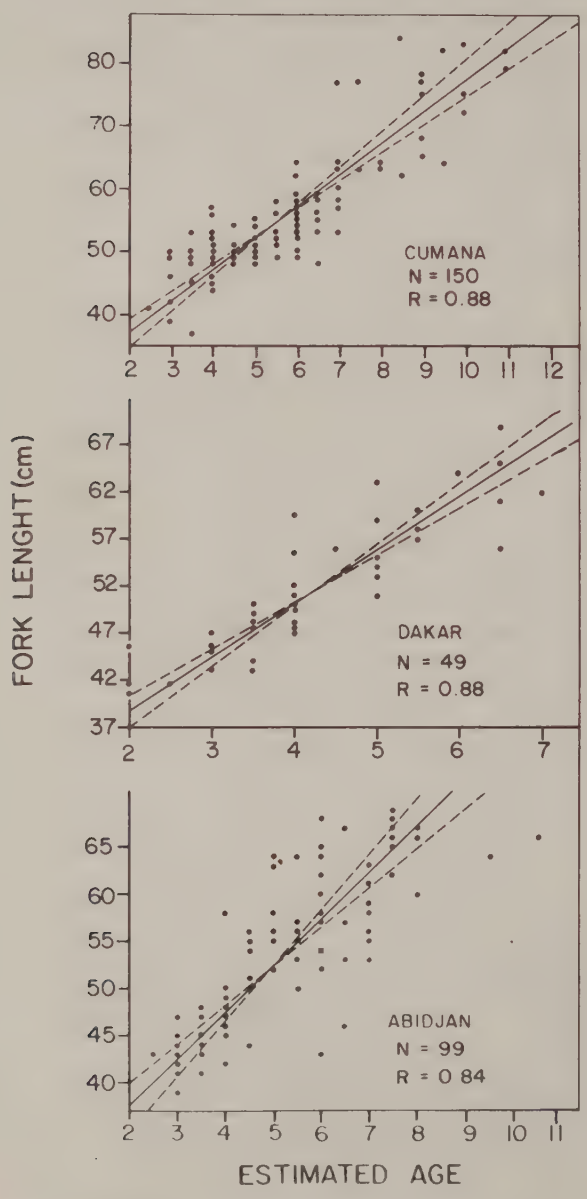

Figure 6.—The relationship between fork length (cm) and estimated age for skipjack tuna sampled at (from top to bottom) Cumana (Venezuela), Dakar (Senegal), and Abidjan (Ivory Coast). Solid lines are functional regressions and dashed lines are the predictive regressions.

We have mentioned that the hypothesis of two rings per year assumed for several other species of tuna was also assumed in this study. We attempted to substantiate this assumption by observing the nature (translucent or opaque) of the edge of skipjack tuna dorsal spine sections from fish landed at Dakar during 1980. The proportion of translucent edges was calculated per month. Figure 7 suggests that from January to June there was a long period of inhibited growth (translucent edge). From July to September, growth appeared to resume (opaque edge), and later in October a new translucent edge appeared. Finally, growth resumed in November and December. This pattern seems to suggest the formation of two rings a year. Nevertheless, several reservations include: 1) Monthly samples were small and did not take into account possible interschool differences or differential growth between sexes (Cayré 1981). 2) The interpretation of the edge of a section is difficult and is highly variable from one reader to another. 3) A period of inhibited growth from January to July seems too long to discount the possibility that several rings may form during this period.

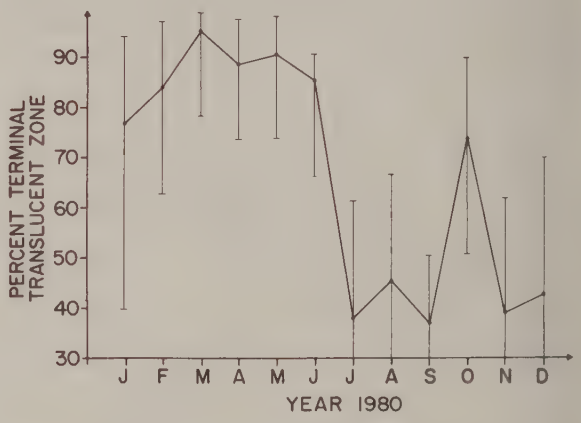

Figure 7.—Percent terminal translucent zone (± range) by month during 1980 for skipjack tuna caught off Dakar, Senegal.

On the basis of annual periodicity, the increments examined in this study (averaging 4 to 5 cm FL between cohorts) are generally two times less than other estimates for Atlantic skipjack tuna based on hardparts (Batts 1972; Carles Martin 1975; Cayré 1979). It seems obvious that our results must be regarded as provisional. The continued research during the ISYP skipjack tuna program should provide additional data on this topic. In particular, tetracycline marking may clarify doubts concerning the time of ring formation and related interpretation of bands on spine sections. Following methodology described by Wild and Foreman (1980), skipjack tuna have been injected with tetracycline during ISYP tagging cruises. The first returns from injected skipjack tuna show that the antibiotic is visible on dorsal spine sections under fluorescence microscopy. The present number of tetracycline marked and recaptured fish (52) and their time at liberty (maximum time: 5 mo for one individual) are not sufficiently large to permit a study of growth at this time. Only fish with at least 1 yr at liberty could validate ring periodicity for the annual cycles and

only for growth during the time each returned fish was at liberty.

In summary, readers of this study have been led to note that: 1) Inhibited growth bands are numerous and may be large, indicating frequent and/or long periods of inhibited growth. 2) Growth bands may also be narrow, indicating short periods of rapid growth. 3) Bands are frequently different from one fish to another (from the same area), which indicates a great variability of individual growth.

These remarks lead us to propose a relatively high growth rate for skipjack tuna which may be related to favorable local environmental conditions. This hypothesis has already been advanced based on gonad maturation studies (Cayré 1981). Although reading skipjack tuna spines to assign ages is a simple and easy method to employ for age estimation, the major difficulties we identified need to be addressed before this method is widely used.

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Otolith Formation and Increment Deposition in Laboratory-Reared Skipjack Tuna, *Euthynnus pelamis*, Larvae

RICHARD L. RADTKE[1]

ABSTRACT

Light and scanning electron microscope techniques were used to examine increments in otoliths of 58 laboratory-reared skipjack tuna, *Euthynnus pelamis*, larvae. Ten larvae were examined daily until day 5, when only eight larvae were alive for sampling. Despite the short survival period, it was possible to validate the time sequence of otolith increment formation. Otoliths were the first calcified tissues formed and calcification appeared to initiate in the otolith core after matrix formation. Increments were first observed 1 d after hatching and continued to be formed at a rate of about one each day, until the experiment ended on day 5. Very little body growth was evident in the reared larvae and they apparently used only yolk-sac nutrients for survival. However, despite the lack of appreciable growth, incremental zones formed in otoliths on approximately a daily basis for the first 5 d of life.

INTRODUCTION

Skipjack tuna, *Euthynnus pelamis*, are abundant in tropical and subtropical waters and compose an extremely valuable fishery resource. However, there is a general lack of data on larval stages of their life history, particularly age and growth. The period between spawning and the first appearance of skipjack in surface waters is of interest since this stage may play an important role in the success (size) of each year class (Seckel 1972). Therefore, an understanding of age and growth of young skipjack is essential for population analysis. To date, however, most research has emphasized older age categories using skeletal hardparts and length-frequency analysis as methods of age estimation (Bell 1962; Le Guen and Sakagawa 1973).

Wild and Foreman (1980) and Uchiyama and Struhsaker (1981) found evidence of incremental growth on otoliths from skipjack tuna, but did not examine young-of-the-year. Age and growth estimates of larval fish are often based on length frequency analysis (Sameoto 1972). However, Brothers et al. (1983) discovered incremental growth bands on the otoliths of field-collected bluefin tuna, *Thunnus thynnus*, larvae which appeared to form on a daily basis for the first 2 wk of life and could be used to estimate age for this time period.

In addition, daily growth bands have been found in otoliths of many species of larval fish. For example, Brothers et al. (1976), Radtke (1978, 1980), Radtke and Waiwood (1980), and Uchiyama and Struhsaker (1981) all found daily growth bands in otoliths of marine teleosts representing numerous families. These studies did indicate that otolith increments formed at different developmental stages but were characteristic of the species being studied. Some species hatch with increments already formed (i.e., mummichog, *Fundulus heteroclitus*, Radtke 1978), while others (i.e., northern anchovy, *Engraulis mordax*, Brothers et al. 1976) do not form increments until yolk sac absorption. By rearing fish larvae under controlled conditions and obtaining otoliths from a time series of specimens of known age, species-specific characteristics of otolith growth can be accurately determined. Recent breakthroughs in hatching and rearing of highly pelagic tunas (Kaya et al. 1981) have allowed such techniques to be applied for the first time. Therefore, the objectives of this research were to examine otoliths from a time series of laboratory-reared skipjack tuna larvae to describe growth characteristics and determine the frequency of increment formation.

METHODS AND MATERIALS

Approximately 10,000 fertilized eggs were obtained from adult skipjack tuna held at the Kewalo Research Facility of the Southwest Fisheries Center's Honolulu Laboratory, NMFS, NOAA, Honolulu, Hawaii. The adult tuna were captured by hook and line and maintained in outdoor circular tanks. The procedures for adult maintenance and fertilized egg acquisition were similar to those applied to the scombrid *Euthynnus affinus* (Kaya et al. 1981).

The culture system for embryos and larvae was based on the design of Hunter (1976). A black fiberglass, cylindrical tank 1.22 m in diameter and 40 cm in depth was immersed in a temperature-controlled bath with the temperature kept around 26°C. Light was supplied by fluorescent lights in accordance with natural photoperiod conditions. The larvae were fed the rotifer *Braebionus plicatus*.

Approximately 5,000 larvae were present at the beginning of the experimental period and 10 larvae were sampled daily throughout the experiment, except only 8 specimens were available by day 5. Only actively swimming, live larvae were sampled for otoliths. The larvae were frozen for storage, and later both sagittae otoliths were dissected from the thawed larvae at 80× under a dissection microscope with the aid of minute insect needles mounted on wooden rods. The dissected otoliths were separated from extraneous organic material, washed with distilled water, dried, and mounted on glass slides with permanent mounting medium. The glass slide mounted otoliths were examined at 1,000× under a compound light microscope to

[1]University of Hawaii at Manoa, Hawaii Institute of Marine Biology, P.O. Box 1346, Coconut Island, Kaneohe, HI 96744.

make increment counts and to measure otolith diameters. Diameters of each otolith were taken from the widest dimension.

Counts of increments on otoliths were based on photographs taken of each fish. I made three counts of each otolith and photographs were randomized between each count. If two of the counts were identical, that increment count was accepted. If none of the counts were identical, the sample was rejected. The use of this counting procedure resulted in a zero rejection rate.

The internal microstructure of skipjack tuna otoliths was examined by fixing the otoliths on a scanning electron microscope (SEM) stub with a 5-min epoxy. Otoliths were polished and ground with 0.3μm alumina paste until the core area was revealed. The polished otoliths were etched with 7% EDTA (disodium ethylenediaminetetraacete, pH 7.4, adjusted with NaOH) for 1 min, coated with gold, and viewed in a SEM.

RESULTS

The sagitta and lapillus were present in skipjack tuna larvae at the time of hatching and were the first calcified tissues to develop. Otoliths of newly hatched larvae did not display increments (Fig. 1), but the core and primordium were observed (terminology of Brothers and McFarland 1982; Tanaka et al. 1981). At this time the otoliths were spherical and the last stages of otolith formation apparently took place during the egg stage, which lasted only 3 d. Growth increments, which were defined as "bipartite structures composed of one optically transparent and one less transparent layer" (Brothers et al. 1983), began to form 1 d after hatching (Fig. 2) and continued at the rate of approximately one increment per day for 5 d (Table 1). No significant difference was detected between the observed number of increments and an assumed increment periodicity of one increment per day (Student's t-test, $\alpha = 0.05$). Therefore, daily increments were validated at the time of hatching for skipjack tuna larvae and during the first 5 d of life.

Skipjack tuna larvae did not demonstrate significant body growth (Table 1) throughout the survival period, which lasted 5 d, as only eight active larvae could be collected on day 5. Furthermore, only a slight increase in otolith diameter could be detected. These data suggest that the larvae were relying primarily on yolk sac nutrients for nourishment, even though food items were available and observed in larval fish stomachs.

Figure 1.—Sagitta otolith from a newly hatched skipjack tuna larva (3.30 mm TL). No increments are present at hatching.

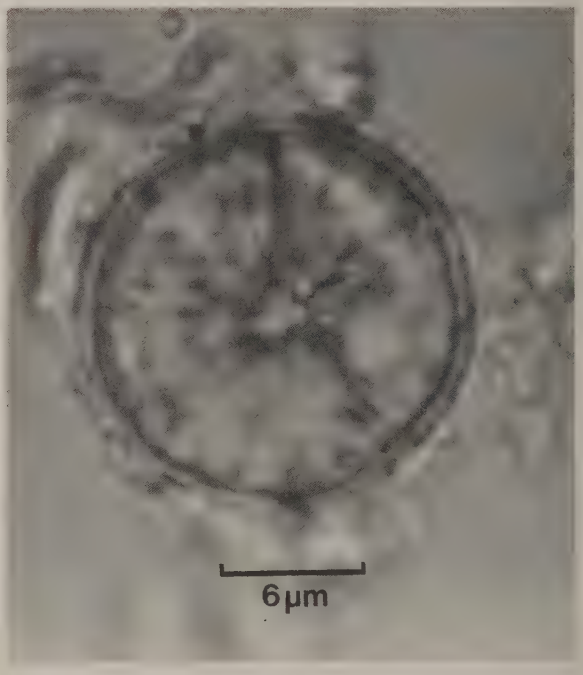

Figure 2.—Sagitta otolith from a 1-d-old skipjack tuna larva (3.50 mm TL) with one increment.

Table 1.—Days after hatching, mean fish lengths, increment counts, and mean sagitta diameter for 58 skipjack tuna larvae reared from eggs.

Age (d)	Sample size	Fish length (mm, TL) (Mean ± SD)	Increment count (Mean ± SD)	Sagitta diameter (μm) (Mean ± SD)
0 (hatching)	10	3.35 ± 0.03	0	18.3 ± 0.6
1	10	3.43 ± 0.06	0.8 ± 0.3	18.9 ± 0.6
2	10	3.52 ± 0.07	1.7 ± 0.3	21.0 ± 0.7
3	10	3.60 ± 0.06	2.9 ± 0.7	22.3 ± 0.5
4	10	3.59 ± 0.10	3.7 ± 0.5	23.0 ± 0.7
5	8	3.60 ± 0.13	4.2 ± 0.8	23.8 ± 1.1

Despite these occurrences, increments were formed on a daily schedule during the survival period. Even larvae with almost no growth in total length (Fig. 3) still displayed daily increment formation, but size of sagittae diameter was proportional to size of larvae (Table 1). Accordingly, increment formation in skipjack tuna otoliths is a reliable indicator of age for at least the first 5 d of life and conceivably longer.

Scanning electron microscope investigations on reared larval skipjack tuna otoliths corroborated the light microscope observations that one increment formed per day after hatching. It was often necessary to perform numerous polishings and etchings in order to reveal the core of the otolith. The rugose surface of the etched otolith provided a detailed image of the otolith increments (Fig. 4). These increments could have been counted easily and it would also have been feasible to measure individual increment width. The SEM techniques are laborious, but they make it conceivable to study otolith microstructure, early growth disruptions, and otolith components as they relate to a larva's past growth history.

Figure 3.—Sagitta otolith from a 3-d-old skipjack tuna larva with three increments (3.50 mm TL). Depsite negligible body growth, increments were still observed.

DISCUSSION

Individual growth is a major indicator of a fish's well-being, and knowledge of larval skipjack tuna growth is a strong index of larval fitness. Furthermore, information on larval growth would provide knowledge of life history strategies and population stratifications. The growth of skipjack tuna larvae is easily studied by examining daily increments found in otoliths

Figure 4.—Scanning electron microscope preparation of a sagitta otolith from a 4-d-old skipjack tuna larva (3.60 mm TL). Four distinct ridges (numbered) were observed originating from the core region.

(Pannella 1971), since the larvae hatch without scales or other usable hardparts. While otolith ageing techniques have been applied to adult skipjack tuna (Wild and Foreman 1980; Uchiyama and Struhsaker 1981), larval stages have not been intensively investigated. Foremost in the investigation of larval skipjack tuna otolith increments is the validation of the time sequence of their formation. Validation would increase the confidence of presumed daily increment counts and establish accuracy of resulting age estimates.

Skipjack tuna larvae began otolith increment formation 1 d after hatching and continued on a daily schedule throughout the 5-d survival period. Otoliths were the first calcified tissue formed and were evident in the embryological stages. Although little information is available on otolith formation in scombrids, otoliths are a prominent and easily observed structure as indicated in scrombrid developmental studies (Sanzo 1932 cited in Brothers et al. 1983; Matsumoto 1958). Recent studies of otoliths in other species of larval fish have shown that, while otoliths are always present at hatching, species differ in the number of increments present at that time. Brothers et al. (1976) found that two to four increments may form prior to hatching in species that have relatively large eggs and long incubation periods, such as the grunion, *Leuresthes tenuis*. Other species with small eggs and short incubation periods, such as the winter flounder, *Pseudopleuronectes americanus*, did not begin to deposit increments on otoliths until yolk sac absorption was complete (Radtke and Scherer 1982). Furthermore, Atlantic cod, *Gadus morhua*, begin increment formation at the time of hatching (Radtke and Waiwood 1980). Skipjack tuna appear to follow the same incremental formation sequence as cod. Therefore, because of the wide disparity in initiation of otolith increment deposition in larval fish, species-specific studies must be conducted to establish the time of first increment formation in each case, if otoliths are to be used as sources of accurate age information.

The larvae in this study hatched at a mean total length of 3.35 mm 3 d after spawning, but appreciable body growth (mean of 3.6 mm TL by day 5) was not found. Matsumoto (1958) surmised that skipjack tuna larvae hatch at 2.5-3.0 mm TL 4 d after fertilization and reach the postlarval stage in 20 d at about 30-40 mm TL. This would approximate a growth rate of almost 2 mm/d. Yoshida (1971) postulated that growth is rapid after metamorphosis and that free-living skipjack tuna attain a total length of 90 mm or grow at about 3 mm/d. Thereafter, growth declines so that skipjack tuna are estimated to be from 31 cm (Joseph and Calkins 1969) to 44 cm TL (Rothschild 1967) at 1 yr of age. In these previous studies, data on the accuracy of age estimates were not presented, and thus questions concerning growth rates of young-of-the-year skipjack still persist. The growth data and short survival time from this study do not conform to previous data and imply that these larvae subsisted mainly on yolk-sac nutrition, and the effects of laboratory rearing negated valid comparisons of growth rates with other studies. Although the data from this study may not provide much insight into growth of skipjack tuna larvae, validation of increments as daily events, despite the general lack of measurable growth, establishes the accuracy of age estimates during this period, with possible extrapolation to field samples given additional data.

Recently, Brothers et al. (1983) counted increments in otoliths of juvenile bluefin tuna (267-413 mm FL) to estimate a mean growth rate of 1.39 mm/d. This research demonstrated the application of counts of otolith increments for estimating early growth and at the same time showed the necessity for validating these experiments, since otolith age was adjusted to absolute age from fertilization by using an assumed correction factor of 4 d. Such an assumption could have a profound affect on age estimates and subsequent growth rate calculations for very young fish, although an error of this type would become progressively less important in older age categories. However, Brothers et al. (1983) did observe two or three diffuse increments, which they postulated to be pre-yolk absorption or prehatching increments. The otoliths from this study also displayed finely spaced increments, which probably resulted from poor growth and a dependence on the yolk-sac for food. In light of these results, I believe this type of otolith data demonstrates potential for determining the time period of yolk-sac absorption and/or periods of nongrowth in larval scombrids and other species. These types of studies would seem to be particularly well suited for SEM techniques.

SUMMARY

The paucity of knowledge on age and growth of larval and juvenile skipjack tuna impedes research of early life history and population biology of this species. Development of an accurate, more direct ageing method, other than size frequency distribution analyses, is essential for advancing future research. The use of daily increments in the otoliths of skipjack tuna larvae increases the resolution of age estimates and promises to provide fishery biologists with a new level of information. When the mechanisms of increment formation are fully understood, the otoliths can be used as a calendar of the early life history for skipjack tuna. Data presented here are the first data available for increment formation in skipjack tuna larval otoliths and confirm that increments began forming 1 d after hatching and continued on a daily basis for 5 d. These data add new insights into the initiation of increments in fish in general and demonstrate that even during no growth or slow-growth conditions, daily otolith increments are still formed. Further research would be necessary, with longer survival times and increased sample sizes, to determine which factors may be important to larval skipjack tuna growth, but otolith studies appear to be one of the most practicable ways to increase the accuracy of age and growth estimations.

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SUMMARY PAPER

Estimating Age and Growth of Little Tunny, *Euthynnus alletteratus*, off the Coast of Senegal, Using Dorsal Fin Spine Sections

PATRICE M. CAYRE[1] and TAIB DIOUF[2]

ABSTRACT

Estimates of age were made from counts of growth bands on dorsal spine sections of 491 little tunny, *Euthynnus alletteratus*, captured off the coast of Senegal during 1979. Analysis of marginal growth bands (by month) indicates that these bands are probably formed during the cold season (November-May). Mean size at estimated age was determined for the first 8 yr of life. These results, though not validated, closely approximated other studies for young fish (estimated ages 1-3), but were highly variable for older age categories. The index of average percent error (*E*) for age estimates from our study was 10.5% and infers good precision.

RÉSUMÉ

La determination de l'age de 491 thonines, *Euthynnus alletteratus*, a été faite par comptage des anneaux de croissance sur des coupes transversales du premier rayon de la nageoire dorsale. L'analyse mensuelle de la nature du bord externe des coupes, indique que les annuli (i.e., zones translucides) se formeraient au cours de la saison froide (novembre a mai). Les tailles moyennes correspondant aux ages de 1 a 8 ans sont données. Ces resultats, bien que non validés par d'autres methodes, sont tres voisins de ceux exposés dans d'autres travaux pour les ages de 1 a 3 ans; des différences non negligeables apparaissent cependant pour les poissons plus agés. L'index de pourcentage moyen d'erreur (E) entre les ages attribués par les deux auteurs est de 10.5% ce qui indique une bonne précision de la methode utilisée.

INTRODUCTION

Commercial tuna fisheries in the eastern tropical Atlantic seem to have reached their maximum sustainable yield for most species (ICCAT 1977-82). Thus, recent economic interest has developed for the less intensively fished little tunny, *Euthynnus alletteratus*, along the Atlantic coast of Africa.

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METHODS AND MATERIALS

Little tunny were collected during 1979 at different commercial landings near Dakar, Senegal, along the Atlantic coast of Africa. We collected data on sex (i.e., males, females, and immature fishes), maturation stage, gonadal weight, fork length (FL), total weight, and obtained the first dorsal spine from each specimen. We attempted to sample all size classes for each sex during each month.

We used the first dorsal spines because they are easy to collect and were often used in many similar studies (Batts 1972; Cayré 1979). In addition, we previously reported (Cayré and Diouf 1981) that dorsal spines of little tunny are good structures to use as a source of age and growth information.

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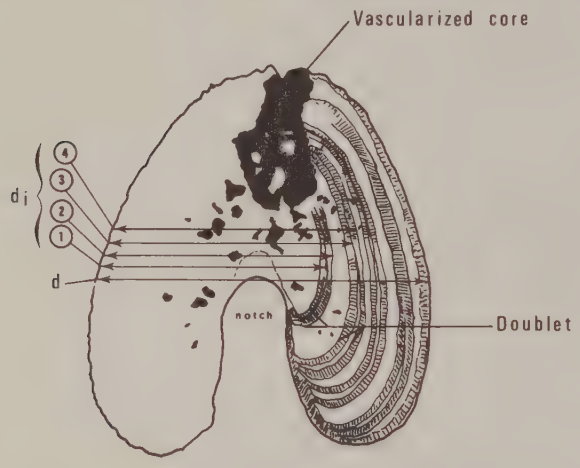

Figure 1.—Cross section of the first dorsal spine of little tunny. Measurements taken: spine diameter (d) and diameter (d_i) of translucent bands (1, 2, 3, 4). The vascularized core, notch, and bands interpreted as doublets (see text) are also shown.

Growth bands observed under the above optical conditions were distinguished by two types of alternating growth zonation; translucent zones, assumed to be indicative of slow growth, were separated by opaque zones, assumed to represent fast growth (see Glossary). We use the term annulus as synonymous with translucent zone (see Glossary). For purposes of assigning an age to each fish, we each read the most anterior of the three cross sections twice by counting translucent zones. When discrepancies occurred, the first section and the other two sections from the same spine were examined by us to arrive at a mutual estimate of age.

Antoine et al. (1983) used a series of coded descriptions of the types of growth bands observed on each section to document how different readers interpreted bands and assigned ages. This methodology was also adopted for our study. The precision (repeatability) of the counts of annuli (translucent zones) was assessed using the method described by Beamish and Fournier (1981). This method uses an index (E) to estimate average percent error:

$$E = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{X_{ij} - X_j}{X_j}\right]100 \qquad (1)$$

where N = number of fishes aged
R = number of times each fish was aged
X_{ij} = ith age estimate of the jth fish
X_j = average estimated age calculated for the jth fish.

The vascularized core (center) of little tunny spines increases in size and complexity with increases in the size and age of fish. In little tunny > 45 cm FL, early growth bands tend to be obscured because of the enlarged core (also see Antoine et al. 1983; Johnson 1983; Compeán-Jimenez and Bard 1983; Berkeley and Houde 1983); fish ≤ 45 cm FL were not affected. Therefore, we estimated the average location of the first annual mark (d_1) from young fishes and used these measurements to determine the number of bands obscured in larger fishes.

The relationships between R and fork length for each sex (male, female, and immature) and for total sample (all sexes combined) were determined with least square regressions and the degree of significance set at $\alpha = 0.05$.

The time of annuli formation was assessed by observing the type (translucent or opaque) and frequency of growth bands occurring on the outside margin of each section. This type of marginal growth (see Glossary) was difficult to determine because the truncated cone shape of sectioned spines caused problems with light diffraction. This difficulty was partially resolved by placing the largest section surface down before microscopic examination. In addition, distinguishing marginal growth was also enhanced by alternately switching the light source from transmitted to reflected light.

RESULTS

Monthly Sample Sizes

Dorsal spines were collected from 497 little tunny (26.4-86.0 cm FL). Cross sections of spines from 491 specimens (239 males, 232 females, 20 immatures) were used in the ageing analysis; six abnormally shaped spines were rejected. Monthly samples ranged from a high of 61 fish in March to a low of 13 fish in December (Fig. 2). Both sexes and the entire size range were well represented in monthly samples, except for the relatively narrow size range of fish collected in January and December (Fig. 2).

Characteristics of the Annulus

We define annuli as translucent bands but the quality of annuli varied considerably among individual fish, ranging from a very well-defined narrow band to a wide diffuse one. The description code we used (see Antoine et al. 1983) allowed us to note all these different types of annuli.

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Due to the complexity of the quality of annuli, a consultation between readers, in order to obtain a mutual interpretation of bands, was necessary for 30% of the spines (147 samples). After this mutual interpretation, an agreement was always reached.

SUMMARY PAPER

Estimating Age and Growth of Little Tunny, *Euthynnus alletteratus*, off the Coast of Senegal, Using Dorsal Fin Spine Sections

PATRICE M. CAYRE[1] and TAIB DIOUF[2]

ABSTRACT

Estimates of age were made from counts of growth bands on dorsal spine sections of 491 little tunny, *Euthynnus alletteratus*, captured off the coast of Senegal during 1979. Analysis of marginal growth bands (by month) indicates that these bands are probably formed during the cold season (November-May). Mean size at estimated age was determined for the first 8 yr of life. These results, though not validated, closely approximated other studies for young fish (estimated ages 1-3), but were highly variable for older age categories. The index of average percent error (*E*) for age estimates from our study was 10.5% and infers good precision.

RÉSUMÉ

La determination de l'age de 491 thonines, *Euthynnus alletteratus*, a été faite par comptage des anneaux de croissance sur des coupes transversales du premier rayon de la nageoire dorsale. L'analyse mensuelle de la nature du bord externe des coupes, indique que les annuli (i.e., zones translucides) se formeraient au cours de la saison froide (novembre a mai). Les tailles moyennes correspondant aux ages de 1 a 8 ans sont données. Ces resultats, bien que non validés par d'autres methodes, sont tres voisins de ceux exposés dans d'autres travaux pour les ages de 1 a 3 ans; des différences non negligeables apparaissent cependant pour les poissons plus agés. L'index de pourcentage moyen d'erreur (E) entre les ages attribués par les deux auteurs est de 10.5% ce qui indique une bonne précision de la methode utilisée.

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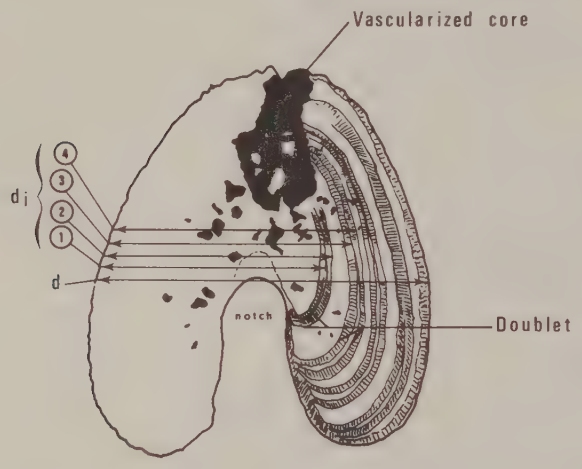

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Season of Annuli Formation

The highest percentage of marginal annuli (terminal slow growth) appears to occur from November to May (Fig. 3), suggesting that annuli are formed during this period. The seasonal occurrence of slow growth (Fig. 3) is inversely related to mean sea surface temperatures (i.e., slow growth appears to occur during periods of low temperatures).

Diouf (1980) reported that the period of maximum spawning for little tunny takes place from June to October. Thus, if annulus formation occurs in November-May, then the age at first annulus formation would be about 6 mo. The wide range in potential slow growth (November-May = 7 mo) makes it difficult to make definitive statements on the time of first band formation. Three females (Table 1) and two immatures, sampled during this period, had only one incomplete and one thin marginal annuli and were estimated to be 0.5 yr. These observations tend to support our statement on the period of the first band formation.

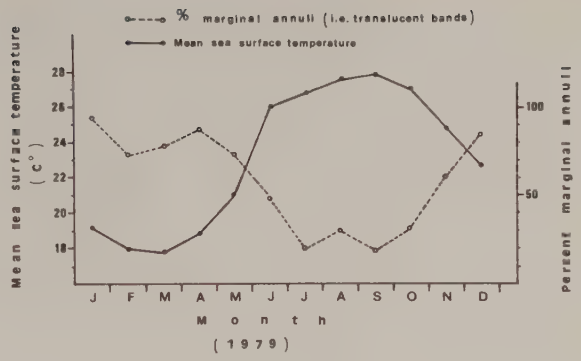
% marginal annuli (i.e. translucent bands)
Mean sea surface temperature
Mean sea surface temperature (c°)
Percent marginal annuli
J F M A M J J A S O N D
Month
(1979)

Figure 3.—Monthly mean sea surface temperatures off the Senegal coast in 1979 (solid line) and corresponding percentages of spine sections of little tunny caught off Senegal, 1979, showing a marginal translucent band (dotted line).

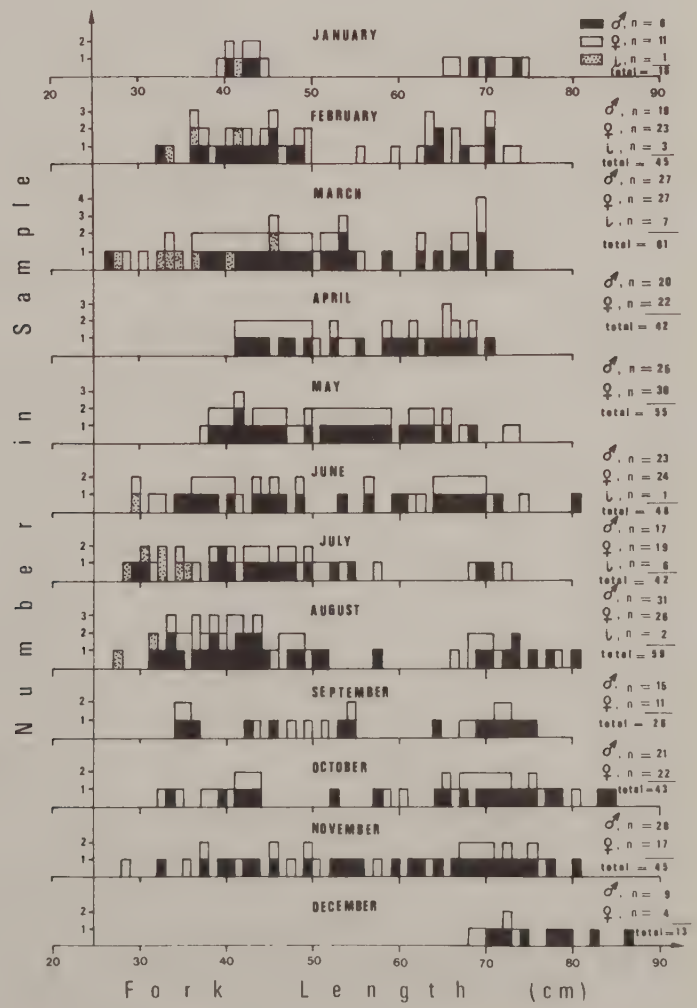

Figure 2.—Fork lengths of monthly samples of 491 little tunny caught off Senegal during 1979.

Table 1.—Total number of little tunny caught off Senegal by size interval (cm FL), number of fishes with obscured growth marks due to enlargement of the vascularized core, and correction rate for obscured growth marks.

Size interval FL (cm)	Total number of fishes	Number of fishes with obscured growth marks			Total correction rate (%)
		1 growth mark	2 growth marks	Total	
46-55	97	22	1	23	24.7
56-65	66	25	1	26	39.4
66-75	115	53	25	78	67.8
> 75	17	3	10	13	76.5
Total	295	103	37	140	47.5

Fish Size-Fin Spine Diameter Relationship

A significant linear relationship (Fig. 4) was found between spine diameter and fork length of female (r = 0.89), male (r = 0.90), and immature little tunny (r = 0.69). However, the small sample and restricted size range of immature fish make the results of this category tentative. A significant linear relationship was found between spine diameter and fork length of all samples (with all sexes combined). This relation was:

$$FL = 0.01456\,d + 16.215 \qquad (r = 0.907)$$

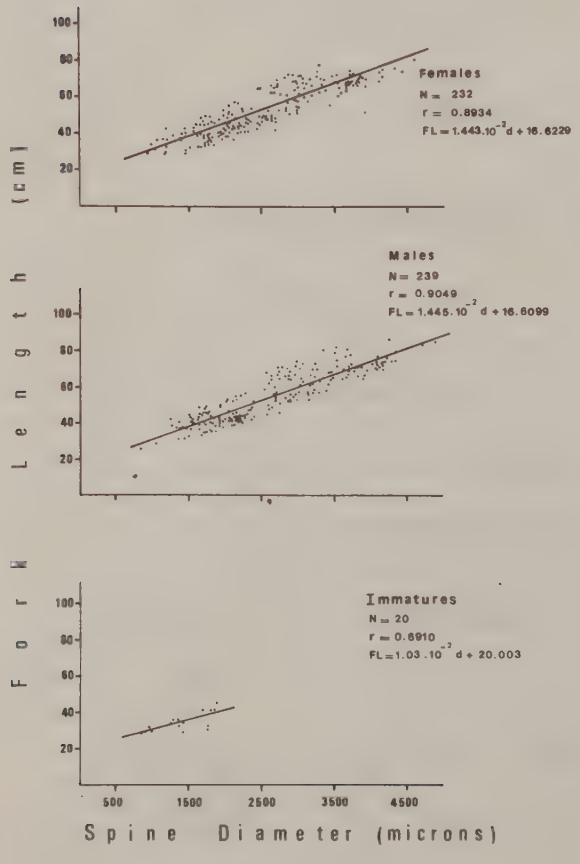

Figure 4.—Relationship between fork length (cm) and first dorsal spine diameter (microns), for females, males, and immature little tunny from Senegal.

where FL = fork length (cm)
d = first dorsal fin spine diameter (μm)
r = correlation coefficient.

Precision of Readings

The average percent error (E) of the counts of annual marks was 10.5%. Differences between our counts exceeded two annual marks for 8% of the samples.

Correction of Assigned Ages

We found that in little tunny < 45 cm FL, it was not necessary to correct for bands obscured due to enlargement of the vascularized core. However, in the 295 fish ≥ 45 cm FL, we had to estimate the number of obscured growth marks. The corrections, which did not exceed two growth marks, were applied to 140 fishes (Table 1). The number of growth marks obscured and needing correction increased with fish size.

Estimated Ages and Mean Fork Lengths

The ranges in mean fork lengths for estimated ages 0.5 through 8 yr were 33.2 to 80.2, 30.1 to 77.2, and 29.4 to 80.2 for males, females, and all samples combined, respectively (Table 2). Relatively small differences were observed for mean size at estimated ages between sexes (Table 2), and the combined sample (males, females, immature) illustrated in Figure 5 shows a progressive increase in mean size and variation about the mean as estimated age increases.

DISCUSSION

Two sources of bias in spine measurements appear to be a direct result of little tunny spine morphology. First, the spine sections were asymmetrical and this irregular shape would have influenced measurements of spine diameter (d) and diameter of annuli (d_i). Secondly, the tapering of the spine could have also affected these measurements. Both sources of bias were not considered to have a significant influence on spine measurements and if they occurred, they probably were consistent between size categories and sexes because of the strong relationship observed between fork length and spine diameter (Fig. 4). The strong relationship between fork length of little tunny and spine diameter also suggests that this structure would be appropriate for use in back calculation of previous growth history.

It was beyond the scope of this study to investigate the cause of annuli formation. The occurrence of maximum slow growth

Table 2.—Estimated ages, corresponding mean fork lengths, interval of fork lengths, and standard deviations (SD) for males, females, and total sample (males, females, immatures) for 491 little tunny caught off the coast of Senegal during 1979.

Estimated age (yr)	Males				Females				Males, females, immatures			
	N	Mean FL	FL intervals (cm)	SD	*N*	Mean FL	FL intervals (cm)	SD	*N*	Mean FL	FL intervals (cm)	SD
0.5	0				3	30.1	28.6-33.0	2.484	5	29.4	27.6-33.0	2.094
1	13	33.2	26.5-36.5	3.218	12	34.3	29.5-44.9	4.057	39	33.4	26.4-44.9	2.249
1.5	14	38.4	32.4-43.3	3.440	21	38.0	32.8-44.0	2.927	38	38.5	32.4-45.0	3.238
2	47	41.8	33.6-52.8	3.730	43	42.0	35.2-49.6	3.810	91	41.9	33.6-52.8	3.730
2.5	14	43.5	40.5-49.5	2.507	16	46.4	39.6-51.5	3.721	30	45.0	39.6-51.5	3.453
3	39	49.6	41.5-62.0	5.327	46	49.6	41.5-61.1	5.129	85	49.6	41.5-62.0	5.186
4	32	58.6	47.7-67.0	6.275	28	58.0	49.7-66.3	6.078	60	58.3	49.7-66.3	6.123
5	30	66.9	52.5-79.5	5.806	25	65.3	52.5-72.5	6.010	55	66.2	52.5-79.5	5.895
6	25	68.9	57.0-78.8	5.333	30	69.5	62.8-76.6	3.311	55	69.3	57.0-78.8	4.257
7	20	73.5	66.0-86.0	4.661	8	72.2	65.5-80.8	4.831	28	73.1	65.5-86.0	4.658
8	5	80.2	75.5-84.8	4.011					5	80.2	75.5-84.8	4.011
Total	239				232				491			

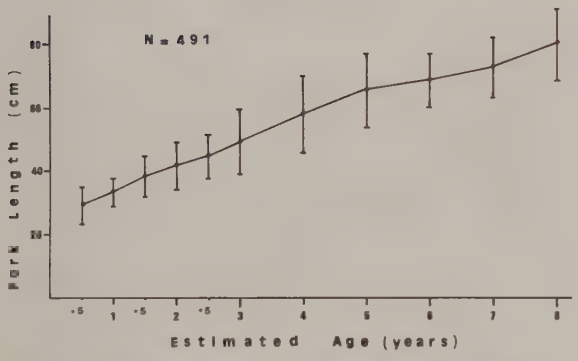

Figure 5.—Estimated age (years) and corresponding mean fork length (cm) ± standard deviation (vertical bars) for 491 little tunny caught off Senegal during 1979.

in spines during months of low water temperatures (November-May) suggests that these factors are related but does not in itself provide an adequate explanation for the variance in number and shape of annuli observed on different fish and also on the same fish. The formation of annuli on little tunny spines probably has several causes, including migration, spawning, and other environmental or biological events that work either in combination or separately to affect the physiology and growth of little tunny. Compeán-Jiminez and Bard (1983) suggested that migration patterns were related to formation of growth bands in spines of eastern Atlantic bluefin tuna, *Thunnus thynnus*, but also acknowledged the shortcomings of their findings. Therefore, more research efforts should be directed towards investigating the cause of annuli formation in scombrids before definitive statements can be made.

The relatively wide range in months when slow growth occurs (7 mo) makes it difficult to pinpoint the exact time of annuli (translucent zones) formation (Fig. 3). There may be several periods of increased and decreased growth from November to May, and this could account for some of the variation in the number and size of the translucent zones observed. Indistinct and narrow translucent zones were often observed in opaque zones near the margin of spines of larger fish. These thin, incomplete annuli appeared to correspond to periods of spawning (June-October as reported by Diouf 1980), but further speculation needs to be tempered by the relatively long duration of the spawning period.

A comparison of mean fork lengths at estimated age from our results with four other studies indicates relatively close agreement for young fish (e.g., estimated age 1 = 34 ± 2 cm), but differences between studies become increasingly more variable in larger fish (Table 3). Age and growth studies of other scombrids (such as bluefin tuna) have also noted close agreement with young tuna and greater disparity in estimates of older age categories (Lee et al. 1983). However, the work on little tunny from the Gulf of Mexico reported by Johnson (1983) was similar to our results through age 6. Johnson's ageing techniques on the dorsal spines were similar to those we used on spines of little tunny from Senegal. Comparisons of our results with others obtained with different techniques by other authors does not constitute validation, but does tend to verify that our results are relatively consistent.

The Beamish and Fournier (1981) method for calculating the average percent error (E) in age estimates was not used in other similar works presented at this workshop; this makes it difficult to compare the precision (repeatability) of our estimates with that of other studies. However, the average percent error (E) we found in our study (10.5%) was smaller than that reported by Antoine et al. (1983), probably because only two readers were involved in our study (compared with eight by Antoine et al.). Cailliet et al. (1983) reported a similar agreement between two readers counting bands on vertebrae from 22 species of sharks.

The increase in number of annuli with size of little tunny from Senegal indicates a strong temporal relationship. However, our interpretation of growth bands on little tunny spines remains unvalidated and this aspect needs to be addressed before further progress can be made.

SUMMARY

1) Spines were collected from 491 little tunny caught off Senegal. They ranged in size from 26.4 to 86.0 cm FL and estimated ages ranged from young-of-the-year to 8+ yr.

2) A strong relationship was observed between fork length and spine diameter. Thus, spines appear to be a good structure for use in back calculating previous growth history.

3) The highest percentage of slow growth bands (translucent

Table 3 – Comparison of mean fork lengths at estimated ages for different studies of little tunny using vertebrae, spines, and length frequencies analysis (Petersen method) to assign ages.

Author	Landau (1965)	Postel (1955)	Rodriguez-Roda (1979)	Cayré and Diouf (1981)	Cayré and Diouf Present study	Johnson (1983)
Method	Vertebrae	Petersen	Vertebrae	First spine	First spine	First spine
Place	Mediterranean Sea	Cap-Vert	Spain	Senegal	Senegal	Gulf of Mexico
Number of specimens	365	983	19	100	491	201
Estimated age (yr)	Mean FL (cm)	FL range (cm)	Mean FL (cm)	Mean FL (cm)	Mean FL (cm)	Mean FL (cm)
0		< 30				
1	35.8	30 to 45		32.9	33.4	35.1
2	53.9	45 to 60	58.1	41.1	41.9	46.2
3	63.7	60 to 75	67.9	49.2	49.6	53.0
4	70.1	> 75	76.2	57.4	58.3	56.1
5	75.5		86.0	65.6	66.2	59.9
6	80.1			73.6	69.3	62.4
7	81.0			77.0	73.1	
8					80.2	

zones) occurred on the margins of little tunny spines in November through May. Although annuli appear to be formed during this period, the wide duration (7 mo) makes it difficult to pinpoint annulus formation in individual fish.

4) The average percent error (*E*) in this study was 10.5% and this was relatively high precision compared with other studies.

5) Annual marks obscured by the vascularized core were estimated in fish ≥ 45 cm FL based on the location and number found in young fishes.

6) Our interpretation of annuli on spines of little tunny from Senegal was similar to other studies for young fish but increased variation was evident in older age categories. However, the accuracy of estimates of age remains unvalidated.

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Comparison of Dorsal Spines and Vertebrae as Ageing Structures for Little Tunny, *Euthynnus alletteratus*, from the Northeast Gulf of Mexico

ALLYN G. JOHNSON[1]

ABSTRACT

The first dorsal spine (cross section) and 33rd caudal vertebra were used to estimate the age and growth of little tunny, *Euthynnus alletteratus*, from the northeast Gulf of Mexico. Spines from 234 fish (315 to 741 mm fork length) and vertebrae from 121 fish were collected off Panama City, Fla., in 1980 and 1981.

Ninety-six percent of the number of growth bands on cross sections of the first dorsal spine agreed with the number of ridge groups found on the vertebrae from the same fish. Mean sizes-at-age that were back-calculated from growth band measurements on vertebrae and spines were similar to the mean sizes-at-age estimated from spines of little tunny from Senegal, West Africa, but were less than the sizes-at-age for fish from other areas.

INTRODUCTION

Little tunny, *Euthynnus alletteratus*, is a pelagic species that occurs throughout the tropical and subtropical Atlantic areas, including the Mediterranean Sea and the Gulf of Mexico. This species is a seasonal migrant in the Gulf of Mexico and is abundant off northwest Florida during the summer. No estimate is available of the size of the stock(s) in the Gulf of Mexico; however, the stock is considered to be quite large (Anonymous[2]).

No information is available on age and growth or on methods to obtain such estimates for little tunny in the Gulf of Mexico. Age estimates have been made for this species off Senegal, West Africa (Postel 1955; Cayré and Diouf 1980, 1983), Spain (Rodríguez-Roda 1979), and in the Mediterranean Sea (Landau 1965). These estimates were developed from growth marks on dorsal spines and on vertebrae and from the length-frequency distributions of the catch.

My objective in this paper was to compare the suitability of dorsal spines and vertebrae as age and growth determination structures for little tunny from the Gulf of Mexico.

METHODS AND MATERIALS

Two hundred and thirty-four little tunny were collected from the commercial fishery off Panama City, Fla., in September and October 1980 and June 1981. The fish ranged in size from 315 to 741 mm fork length (FL). The first dorsal spine was removed and the fork length was measured. The caudal peduncle, which included the 33rd vertebra, was collected from 121 of the 234 fish.

Cross sections were prepared from the first dorsal spine by: 1) Sawing the first 3 mm of spine shaft above the condyle with a Dremel[3] tool, 2) placing the shaft section on a mounting tag using Lakeside No. 70C thermoplastic cement and sectioning the shaft using the method described by Berry et al. (1977), 3) removing three 0.18 mm thick serial sections from the cement with 50% isopropanol, and 4) mounting the clean sections in 20% Piccolyte cement (20% Piccolyte, 80% xylene) on glass slides.

Spine cross-sections were examined and measured using closed-circut television, which projected an image of the section onto a monitor screen at 40× magnification. Sections were viewed under transmitted light and measured with a ruler. Translucent (light) ring groups consisting of many fine concentric lines on the cross-sections were counted and their distances from the center of the spine measured following the description by Jolley (1977). These measurements (in millimeters) include the following: 1) Spine radius (R)—the maximum lateral distance at a 90° angle to the spine axis on the largest of the lobes from the estimated center of the spine, and 2) spine radii (B)—the distance from the estimated center of the spine to the distal edge of each incremental growth mark or band (Fig. 1). Each radius was assumed to represent a year-mark.

The vertebrae of the caudal peduncle of each fish were removed and stained using the alizarin red S process of Berry et al. (1977). The 33rd vertebra was examined for growth cycles. This vertebra was selected because its unique shape facilitated its identification and was used by Landau (1965) in her study of little tunny from the Mediterranean Sea.

The vertebrae were cut in half through the dorsal-ventral plane to expose growth marks which appeared as stained ridges on the centrum surface. Ridges on the anterior centrum were counted and measured on the left lateral surface. The counts and measurements were made with an ocular micrometer in a binocular dissecting microscope at 6× magnification. Measurements were as follows: 1) Vertebral cone depth (V)—the distance from the cone focus to the anterior cone edge, and 2) the centrum ridge radii (v)—the distance from the cone focus to the distal edge of each couplet of cone ridges which constituted a presumed year-mark (Fig. 2).

The relationships between R and FL and between V and FL were determined with least square regressions and the degree of significance set at $\alpha = 0.05$. These relationships were used

[1]Southeast Fisheries Center Panama City Laboratory, National Marine Fisheries Service, NOAA, 3500 Delwood Beach Road, Panama City, FL 32407-7499.

[2]Anonymous. 1981. Fishery management plan for coastal migratory pelagic resources (mackerels). Unpubl. rep. Gulf of Mexico and South Atlantic Fishery Management Councils, 5401 W. Kennedy Blvd., Tampa, FL 33609.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

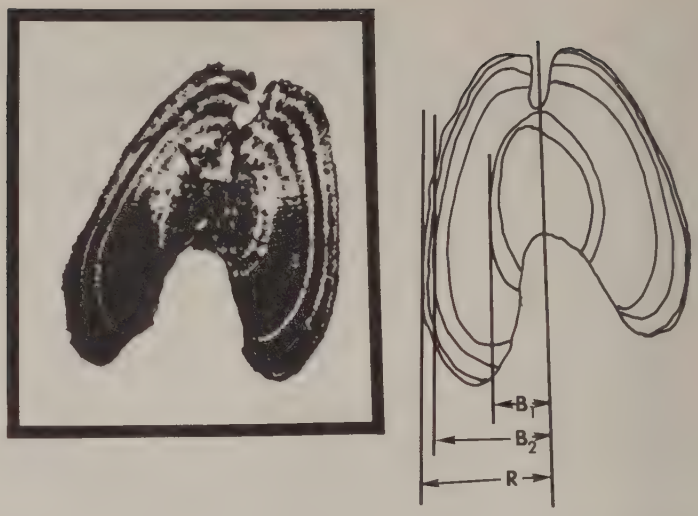

Figure 1.—Cross section of first dorsal spine of a 550 mm FL little tunny collected 2 June 1981 off Panama City, Fla. R is spine radius and B_1 and B_2 are measurements from the center of the spine to the distal edges of spine bands 1 and 2, respectively.

to back calculate the size at band and ridge couplet formations (ages) using methods adapted from Tesch (1971), Ricker (1975), and Everhart et al. (1975).

RESULTS AND DISCUSSION

The first dorsal spine of little tunny is bilobed with a vascularized core or internal matrix (Fig. 1). This core appears to become larger as the fish grows and in older fish may obscure early growth marks (i.e., those close to the spine center).

The appearance of the spine cross sections was similar to that described by Cayré and Diouf (1980, 1983) for little tunny from Senegal. Growth marks (translucent rings were very evident in the cross sections. Cayré and Diouf (1983) reported that these rings were formed in pairs on a yearly basis and referred to them as doublets. The marks on spines in my collection also appeared to be formed in pairs; however, the space between members of a band (doublets) varied and, in some cases, the band appeared to be a wide, single, translucent ring. These wide, single, translucent rings were also counted as year-marks.

A significant relationship that was found between FL and spine radius (R) was expressed best by a power function whose coefficient of correlation (r) was 0.932 (Fig. 3). This equation was $FL = 32.42(R^{0.7135})$.

The back-calculated mean lengths at band formation (estimated ages) were less than parallel to the empirical mean lengths (mean lengths-at-capture), which was expected considering that some growth had occurred between time of band formation and capture (Table 1, Fig. 4).

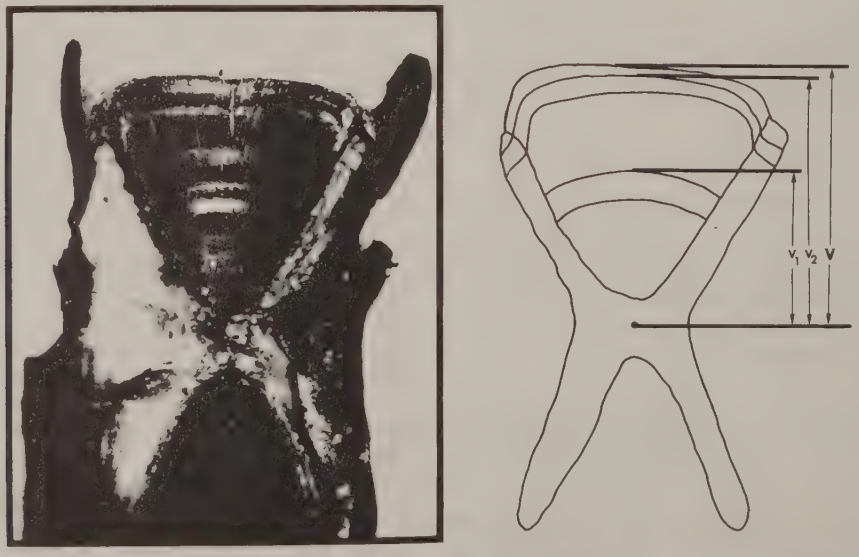

Figure 2.—Vertebral centrum from a 550 mm FL little tunny collected 2 June 1981 off Panama City, Fla. V is vertebral cone radius and v_1 and v_2 are measurements from the vertebra's center to the distal edge of vertebral ridge couplets 1 and 2, respectively.

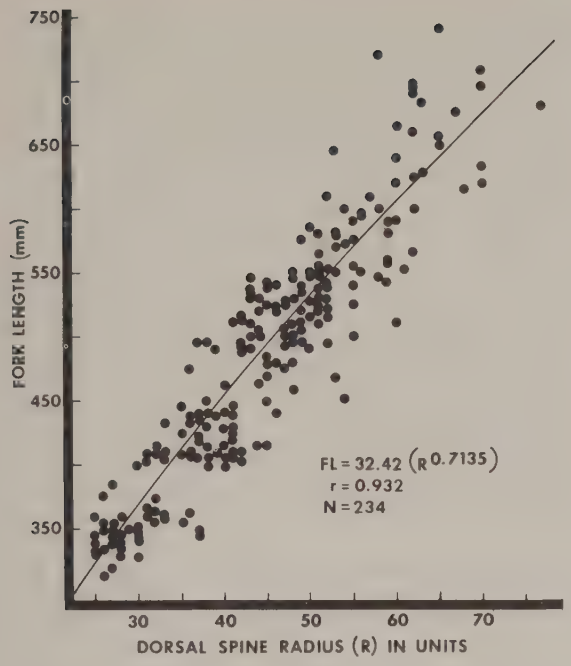

Figure 3.—Relationship between dorsal spine radius and fork length of little tunny from northwest Florida.

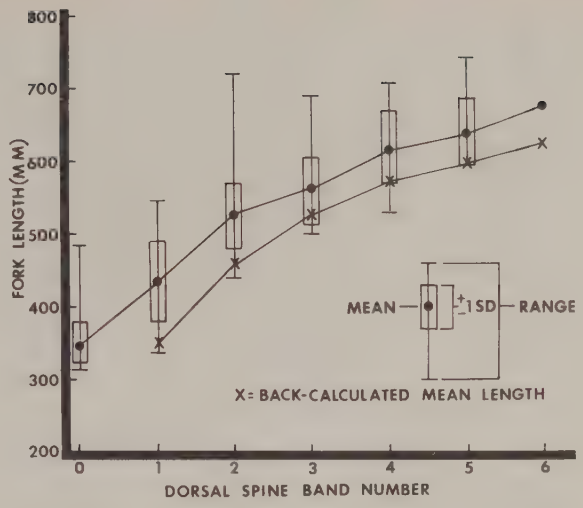

Figure 4.—Length at capture and back-calculated length at spine band formation for little tunny from northwest Florida.

The growth marks in the vertebrae of little tunny are in the form of ridges. The centra of little tunny in the Gulf of Mexico have the same appearance as the centra of little tunny in the Mediterranean Sea described by Landau (1965). The ridges of the centra appear to be formed in pairs or couplets.

A significant relationship was found between FL and vertebral cone radius (V) and was described best by the power function $FL = 44.68(V^{0.6145})$ with $r = 0.863$ (Fig. 5). The back-calculated mean lengths at ridge formation and their corresponding mean back-calculated lengths based on spine band measurements were less than the mean empirical lengths (Table 2, Fig. 6). However, the mean lengths based on spines were consistently longer than their respective mean lengths based on vertebrae, which could indicate that the vertebral ridge couplet formation is completed before the spine band completion. These differences may be the result of differential response of the structures to mineralization (i.e., calcium metabolism) such as was reviewed by Simkiss (1974). One cannot, however, rule out the possibility that the differences are artifacts that are the result of small sample sizes.

An agreement of 96% (3 vertebrae and 2 spines of the 121 pairs were unreadable) was obtained between the estimated age of fish determined by spine bands and the estimated age determined by vertebral ridge couplets. Therefore, both structures appeared to be useful as age and growth estimators for little tunny from the northeast Gulf of Mexico; however, vertebrae seemed to estimate smaller sizes at age than spines.

Other investigators have reported the use of spines and vertebrae to estimate the age of little tunny. Spines were reported by Cayré and Diouf (1980, 1983) as useful structures for age determination of fish from the west coast of Africa, and Rodríguez-Roda (1979) reported their usefulness in ageing little tunny off the Atlantic coast of Spain. I have summarized their information, along with Postel's (1955) age information,

Table 1.—Mean back-calculated fork lengths (mm) at spine band formation for little tunny from northwest Florida.

Band class	Number of fish	Mean length-at-capture (mm)	Mean back-calculated fork length for each band number					
			1	2	3	4	5	6
1	99	437.62	347.31					
2	45	523.36	352.56	458.07				
3	31	560.90	367.69	466.94	532.35			
4	18	612.78	345.44	471.22	533.69	577.05		
5	7	639.71	333.17	450.61	511.90	557.36	602.01	
6	1	675.00	294.99	380.47	467.16	524.13	578.71	623.89
Weighted mean			350.71	461.81	529.71	560.71	599.09	623.89
N			201	102	57	26	8	1
Growth increment			350.71	111.10	67.90	40.00	29.38	24.80

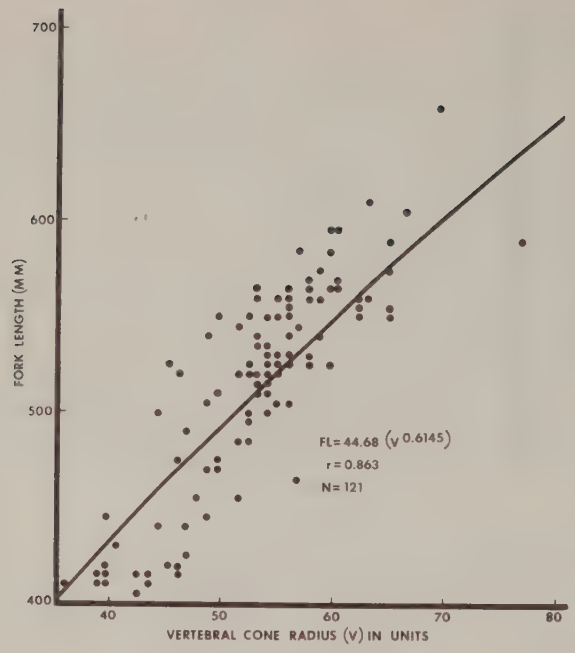

Figure 5.—Relationship between vertebral cone radius and fork length of little tunny from northwest Florida.

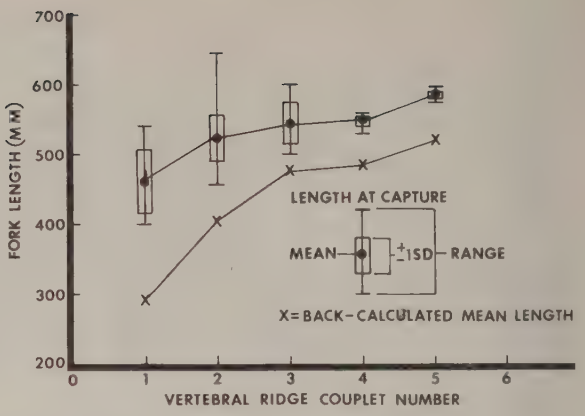

Figure 6.—Length at capture and back-calculated lengths at growth increment count formation and corresponding spine band formation for little tunny from northwest Florida.

developed from length-frequency distributions from Senegal (Fig. 7).

A wide range of back-calculated body sizes at various ages is evident from a comparison between the studies. The mean sizes-at-age for little tunny from the northeast Gulf of Mexico are similar to those reported by Cayré and Diouf (1980, 1983) for fish from Senegal, but they are less than the sizes-at-age reported for fish collected by other investigators.

The differences in the mean sizes at age of the various reports may be the result of differences in racial characteristics or har-

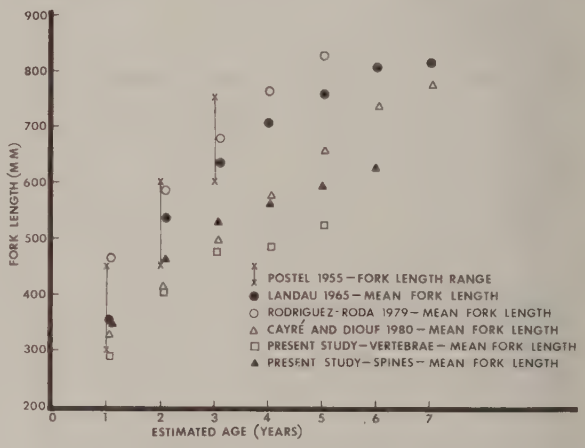
FORK LENGTH (MM)
ESTIMATED AGE (YEARS)
POSTEL 1955 – FORK LENGTH RANGE
LANDAU 1965 – MEAN FORK LENGTH
RODRIGUEZ-RODA 1979 – MEAN FORK LENGTH
CAYRÉ AND DIOUF 1980 – MEAN FORK LENGTH
PRESENT STUDY – VERTEBRAE – MEAN FORK LENGTH
PRESENT STUDY – SPINES – MEAN FORK LENGTH

Figure 7.—Summary of age-length information on little tunny.

Table 2.—Mean back-calculated fork lengths (mm) at vertebral ridge group formation (corresponding values calculated from dorsal spine measurements in parentheses) for little tunny from northwest Florida.

Ridge group class	Number of fish	Mean length-at-capture (mm)	Mean back-calculated fork lengths for each ridge group				
			1	2	3	4	5
1	47	461.98	335.94				
			(378.07)				
2	40	522.78	273.30	432.12			
			(360.78)	(470.78)			
3	24	546.00	246.05	372.22	483.10		
			(348.37)	(441.73)	(503.56)		
4	3	550.67	247.50	328.32	463.80	498.35	
			(320.99)	(395.30)	(442.51)	(492.42)	
5	2	587.50	205.05	348.50	422.44	461.45	520.20
			(340.35)	(429.64)	(488.78)	(533.59)	(584.87)
Weighted mean			291.20	404.35	476.92	483.59	520.20
			(364.09)	(456.20)	(496.22)	(508.89)	(584.87)
N			116	69	29	5	2
Growth increment			291.20	113.15	72.57	6.67	36.61
			(364.09)	(92.11)	(40.02)	(12.67)	(75.89)

vesting techniques. These factors, which influence our perception of stock conditions of little tunny, have been summarized by Yoshida (1979). The present study and that of Cayŕe and Diouf (1980) had few fish older than 4 yr of age and thus probably do not accurately reflect the mean sizes-at-age for older fish in their respective geographic locations.

The generally accepted criteria for validation of a fish's hardpart for age determination and back calculation of size at previous ages are as follows: 1) The hardpart must be constant in number and identity throughout the life of the fish, 2) the hardpart must grow proportional to the growth of the fish, 3) the hardpart must have a recognizable pattern of growth marks, and 4) the hardpart pattern must be such that a regular time scale can be allocated to the pattern (Williams and Bedford 1974; Everhart et al. 1975; Brothers 1983; Smith 1983). The first dorsal spine and 33rd vertebra of little tunny both fit most of the criteria for acceptable age determination and back-calculation structures for this species in the Gulf of Mexico.

The first dorsal spine and 33rd vertebra are unique structures and are easily identified. Both structures have good correlations to fork length ($r = 0.932$ for spines and $r = 0.863$ for vertebrae). Their respective growth patterns (bands on spines and ridge couplets on vertebrae) are formed in a recognizable pattern and agree with each other in number. The final criterion of known mark formation periodicity has not been determined.

Further investigation is needed in the northeast Gulf of Mexico on the age and growth of little tunny, especially to determine the time and cause of mark formation (bands on the dorsal spine and ridges on vertebrae).

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Determining Age of Young Albacore, *Thunnus alalunga*, Using Dorsal Spines

A. GONZÁLEZ-GARCÉS and A. C. FARIÑA-PEREZ[1]

ABSTRACT

The age and growth of albacore, *Thunnus alalunga*, from the North Atlantic, was studied by examining growth bands on cross sections of the first dorsal spine. A total of 266 albacore were collected by commercial trollers in 1980 and 1981 and ranged in size from 38 to 100 cm fork length (FL). Most individuals were sexually immature and measured < 90 cm FL. A significant linear relationship was found between the radius of spine sections (R) and fork lengths ($r^2 = 0.97$). Equations were developed to convert measurements of spine lengths into fork lengths.

Two growth bands appeared to be formed each year on spines from immature albacore. Back calculations of previous growth history were made from measurements of growth bands on spines, and albacore were observed to reach an average length of 39 cm FL in their first year of life. Estimates of fork length from back calculations were fitted to the von Bertalanffy growth model by the Ford-Walford method and estimated parameters were: $k = 0.129^{-1}$; $L_\infty = 140$ cm FL; $t_0 = -1.57$ yr.

RESUMEN

La edad y crecimiento del atún blanco, *Thunnus alalunga*, del Atlántico Norte, fue estudiada examinando las bandas de crecimiento que se pueden observar en cortes de la primera espina de la primera aleta dorsal. Se analizaron 266 atunes blancos procedentes de la pesquería de currican en 1980 y 1981, con un rango de tallas de 38 a 100 cm longitud furcal (LF). La mayoriá de los individuos eran sexualmente immaturos y medían menos de 90 cm. Se encontró una significativa relación lineal entre el radio de los cortes (R) y la longitud furcal del pez ($r^2 = 0.97$). Se construyeron ecuaciones para relacionar la longitud de las espinas con la longitud furcal.

Se observaron dos bandas de crecimiento por año en las espinas. Midiendo el radio de las bandas de crecimiento y usan do retrocálculo se obtuvo que los atunes blancos alcanzan 39 cm LF en su primer año de vida. Estimaciones de la longitud furcal a cada año obtenidas por retrocálculo se emplearon para obtener, mediante el método de Ford-Walford los parametros de la ecuación de crecimiento de von Bertalanffy: $k = 0{,}129^{-1}$; $L_\infty = 140$ cm LF; $t_0 = -1{,}57$ años.

INTRODUCTION

Albacore, *Thunnus alalunga*, are widely distributed in the Atlantic Ocean from lat. 51°N to lat. 40°S. There are two well-defined stocks, one in the Northern Hemisphere and the other in the Southern Hemisphere (Bard 1974). The North Atlantic stock, which is studied in this paper, has its spawning area in the Sargasso Sea and near the north Venezuelan coast (Ueyanagi 1971). Individuals are reported to migrate extensively and these routes change depending on whether or not they have reached sexual maturity (Bard 1974). Young individuals, those that have not reached sexual maturity (< 85 cm fork length, FL), live during the winter in the central North Atlantic, more or less scattered in surface waters. In the spring, the albacore start gathering in thermal front areas that rise up to the north from Madeira and the Azores, and the migration follows these fronts towards the northeast as far as the Bay of Biscay and the south of Ireland. They remain there until mid-September, when young fish returned to the central North Atlantic (González-Garcés 1975.)

When albacore reach sexual maturity, summer migratory routes change and instead of going east towards the Bay of Biscay, they migrate in a westerly direction to spawn in the Sargasso Sea and northern Venezuela (Bard 1974). These spawning areas are usually warm; temperatures often exceed 24°C, with a weak thermal gradient between the surface and 300 m depths (Richards 1969).

After spawning, they return to the central North Atlantic where they spend the winter (González-Garcés 1975). This second type of migration is repeated every year throughout their life. An important characteristic of this species is that at the time they reach sexual maturity, the gas bladder becomes completely developed and allows them to occupy a greater depth distribution in the water column (Bard 1982).

This migratory behavior is reflected in the fishing industry. Young albacore are caught in the summer by surface fleets (trolling and bait boat) in the eastern North Atlantic. Adult individuals are caught at depths of 50 to 150 m by longline, in the central Atlantic in winter and in the western North Atlantic in summer (Bard 1974). Recently, the production has been about 50,000 metric tons (t) annually: 30,000 t by surface gear and 20,000 t by longline (ICCAT 1980).

The relation between length and age is important in growth studies. Several authors have used different methods to determine age of albacore in the Atlantic. Priol (1945), Le Gall (1950, 1952), Aloncle and Delaporte (1976), and Beardsley (1971) used Petersen's method of interpretation of length-frequency data to estimate age. Figueras (1957) studied growth by counting the vertebral rings and Yang (1970) used scales. Bard (1974) and Hue (1980) used both length-frequency and scales. Bard and Compeán-Jimenez (1980) used spines of the first

[1]Instituto Español de Oceanografía, Apartado 130, La Coruña, Galica, Spain.

dorsal fin. Although numerous attempts to age Atlantic albacore have been made using various techniques, there is a general lack of arrangement among the studies (Table 1).

This study was conducted to estimate the age and growth rates of young albacore caught in the northeast Atlantic from thin sections made at the base of the first dorsal spine. Spines were chosen because of the advantages they present compared with other hardparts. For example, spines are easily accessible for sampling since their extraction does not interfere with the market value of the fish. In addition, a higher percentage of spines than scales can be read (51% for scales, Hue 1980, and 96% for spines in this paper). Growth characteristics of spines are also stable throughout the entire life cycle of albacore, while scales can be regenerated.

METHODS AND MATERIALS

Albacore were obtained from the surface fishery (by trolling) of the Spanish tuna fleet in the Bay of Biscay from June to September of 1980 and 1981. Date and geographical area of capture, as well as fork length (FL), were noted for each specimen, but sex was not determined because the majority of fish was immature. The first two spines of the first dorsal fin were sampled, using the method explained by Compeán-Jimenez (1980). A series of three cuts 0.5 mm thick were made with a low-speed Isomet[2] saw and diamond-bearing blades.

To locate the best area for counting the rings that appear in the cross sections, we made a series of cuts along the length of the spine beginning at the condyle base (Fig. 1A). We observed the cross sections with a binocular lens under transmitted light, and then in a profile projector, which permits two observers to view simultaneously. In general, a cross section observed with transmitted light appears with a succession of alternating translucent and opaque bands (see Glossary). The central area (core) of the cross section is occupied by vascularized bony tissue, which complicates interpretation of early growth bands.

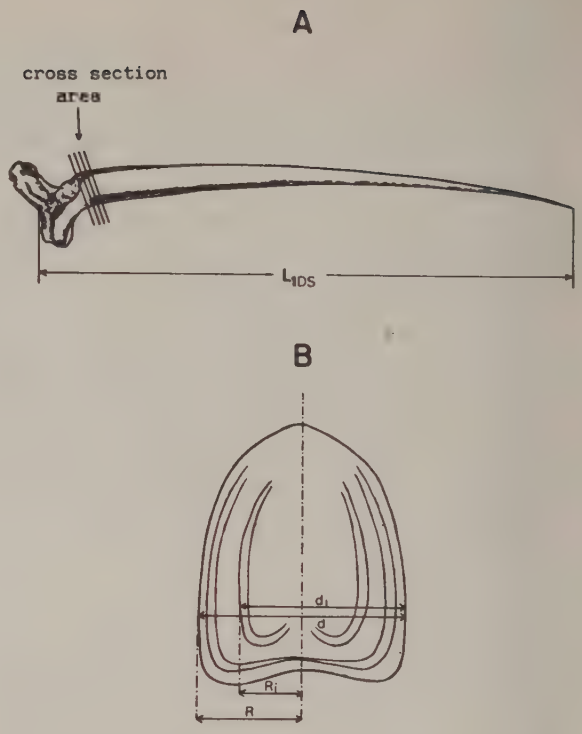

Figure 1.—First dorsal spine and the location of cross sections (A); and cross section of the first dorsal spine showing growth rings and measurements taken (B). R = radius of spine, R_i = radius of ring, d = diameter of spine, d_i = diameter of ring i.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1.—Size-age relation of North Atlantic albacore estimated by different authors and methods of ageing. Sizes refer to fork length in centimeters, except for Priol, Le Gall, and Figueras, where they refer to total length.

Estimated age	Priol (1945) Size freq.	Le Gall (1950) Size freq.	Le Gall (1952) Size freq.	Figueras (1957) Vertebrae	Yang (1970) Scales	Beardsley (1971) Size freq.	Bard (1974) Size freq. Scales	Aloncle and Delaporte (1976) Size freq. Ch.[1]	Cl.[2]	Az.[3]	Hue (1980) S. freq. + scales Cl.[2]	Az.[3]	Bard and Compeán-Jimenez (1980) Spines ♂	♀
1	50-58	25-46	44	17-18	20.4	44	29.5	42.9	40.5	48.5	40.8	48.3	50	
2	59-74	46-60	52	31-32	39.6	55	48	55.3	53.0	61.0	54.5	61.0	63	
3	74-86	60-74	63	44-45	56.1	64	62	65.8	63.5	71.5	63.1	73.5	74	
4	86-94	74-88	75	56-57	71.2	75	74	74.9	73.5	81.0	72.5	83.5	84	
5	94-98		85	69-70	80.9	87	84		79.5				94	
6				81-82	90.3	95	92						104	101
7				91-93	98.1	100	99						107	103
8						104	105						110	
9						108	110							
10						112	114							
11							117							
12							120							
13							122							
Von Bertalanffy parameters														
k					0.19	0.141	0.183						0.2284	
L_∞					135	140	134.4						124.74	
t						−1.63	−0.35						−0.9892	

[1]Ch: "Chicaneurs" subpopulation.
[2]CL: "Classique" subpopulation.
[3]Az: "Acoriens" subpopulation.

In this paper, we refer to each translucent band as a "ring." Translucent bands were counted as rings if they were continuous around the circumference of the entire spine section (Fig. 1B). Two rings typically appeared together (as double rings) where the distance between them was less than the distance to the preceding and following rings (Fig. 1B). We therefore assumed this occurrence to represent two bands per year (discussed in more detail later), and used this interpretation to assign ages. Occasionally, auxillary rings (either in pairs or singular) were encountered that did not extend around the entire cross section; these were considered false rings and were ignored.

For age determination and growth studies, the number of rings (translucent zones) in each cross section of the spine in every individual was counted in order to assign an estimated age and build a size-age key. The counting of rings for each specimen was made once separately by each author. When there was disagreement between counts of rings, spines were read simultaneously. If agreement could not be reached, questionable spines were disregarded.

To estimate the center of the spine and avoid errors resulting from the vascularized core (Fig. 1B), measurements were made with a binocular microscope under 10× magnification from the outside edge of each ring to the opposite edge of the cross section (d_i) following Cayré and Diouf (1981). These distances were converted into radii (R_i) by the formula:

$$R_i = d_i - d/2 \qquad (1)$$

where R_i = radius of the ring i (distance between the center of the cross section and the outside edge of ring i),
d_i = distance from the outside edge of ring i to the opposite edge of the cross section,
d = diameter of the spine (distance from the edge of the cross section to the opposite edge of the cross section).

Radius of the spine was defined as the distance between the estimated center of the cross section (Compeán-Jimenez and Bard 1983) and the edge of the section.

In order to use back calculations to estimate previous growth history, we studied the relationship between the radius of the spine (R, or distance in 0.1 mm, which separates the center of the cross section and the edge) and fork length using regression analysis. Regressions were tested at a significance level of $\alpha = 0.05$.

The fork length of the fish was back-calculated for each one of the different rings, using the formula:

$$FL_i = a + (FL - a)\, R_i / R^* \qquad (2)$$

where FL_i = fork length of the fish corresponding to age or ring i in cm,
a = ordinate in the origin of the equation FL fl $a + bR$,
FL = fork length of the fish in cm,
R_i = radius of the ring i (in 0.1 mm) calculated as the average value observed in ring i,
R^* = median radius of the spine for each size in 0.1 mm.

These fork lengths, back-calculated for each estimated age, were used by the Ford-Walford method to fit the von Bertalanffy growth model and obtain vital parameters.

The length of the first two spines of the dorsal fin and the fork length of the fish were examined to provide a means of converting spine length to fork length because lengths from each fish were not always available, but spines were. The spine lengths were taken in millimeters and the fork lengths in centimeters.

RESULTS

Spines from 266 specimens ranging in size from 38 to 100 cm FL were examined. There was 85% agreement between our counts of growth bands, and subsequent mutual readings improved this to 96%. Mutual agreement between us could not be reached for 4% (10) of the specimens and these were disregarded for analysis.

Based on our own observations of the cross section of the first and second dorsal spines, we found that young albacore (< 100 cm FL) appeared to form two growth bands every year. Double annulation in albacore scales and spines was cited by some authors in previous reports (Yang 1970; Bard 1974; Bard and Compeán-Jimenez 1980; Hue 1980) and also for other scombrids. These two well-defined rings per year were seen in most of the cross sections (Fig. 1B), although occasionally there were also less clearly defined translucent bands that we considered "false" rings. The well-defined rings from the cross section of the second spine were used to verify any doubtful information. Based on this interpretation, a size-age (estimated) key for 256 albacore grouped in size classes of 2 cm is given in Table 2.

The spawning season for this species comprises an extensive period in spring-summer (Yang 1970; Bard 1974; Bard and Compeán-Jimenez 1980). Accordingly, the first ring appears to be formed in fall-winter and the second in spring-summer. The formation of this spring-summer ring completes the first pair of rings. Using these criteria for interpretation, we observed individuals collected in June through September with an even number of rings, i.e., with whole years completed ($n+$), and individuals with an odd number of rings, i.e., with an additional winter's growth band ($n + ½$). In June, 42% of the individuals studied had an even number of rings and 58% an odd number (Fig. 2). These percentages were inverted in July and the percentage of individuals with an even number of rings increased with the advance of summer (Fig. 2). Therefore, we felt the formation of the first ring of the pair occurred between fall and the following spring.

A significant linear relationship was found between spine radius (R) and fork length (FL = 16.341742 + 2.842278 R, r^2 = 0.97, n = 245, Fig. 3). Thus, we felt justified in making back calculations of previous growth from our measurements on spine sections. The mean measurements for the radius of each ring and standard deviations and sample sizes are shown in Table 3. The adjusted Ford-Walford relationship between l_t and l_{t+1} was $r^2 = 0.99$, the vital parameters for the von Bertalanffy growth model were: $k = 0.129^{-1}$; $L_\infty = 140.08$ cm FL: $t_0 = -1.57$ yr, and the equation was: $l_t = 140.08\ [1 - \exp 0.129\ (t + 1.57)]$ (Fig 4).

For the first dorsal spine, 266 pairs of spine lengths ($L_{1_{DS}}$) and the corresponding fork lengths (FL) were used to obtain the equation: FL = 4.806236 + 0.759063 $L_{1_{DS}}$ ($r^2 = 0.96$). In addition, 257 pairs for the second spine ($L_{2_{DS}}$) and fork lengths

Table 2.—Size-age (estimated) key of the 256 albacores studied. The fork length (FL) is regrouped in classes of 2 cm (to the cm lower pair).

Fork length class (cm)	Estimated age (yr)												
	1+	1.5+	2+	2.5+	3+	3.5+	4+	4.5+	5+	5.5+	6+	6.5+	7+
38	2												
40	2												
42	6	1											
44	6	1											
46		3	7										
48		10	4										
50		6	11										
52		2	12										
54		1	13										
56		1	4	5	2								
58			3	9	4								
60			2	2	8	1							
62				6	2	1							
64				4	6	1							
66					6	2							
68					3	5	2						
70					1	4	6						
72						9	8						
74						2	8	1					
76						2	4	2	1				
78								3					
80							3		2				
82									9				
84							1	2	4	1			
86							1	1	3		1		
88										1	1		
90									1	1	2		
92										1	1		
94													
96													
98											1		1
100												1	

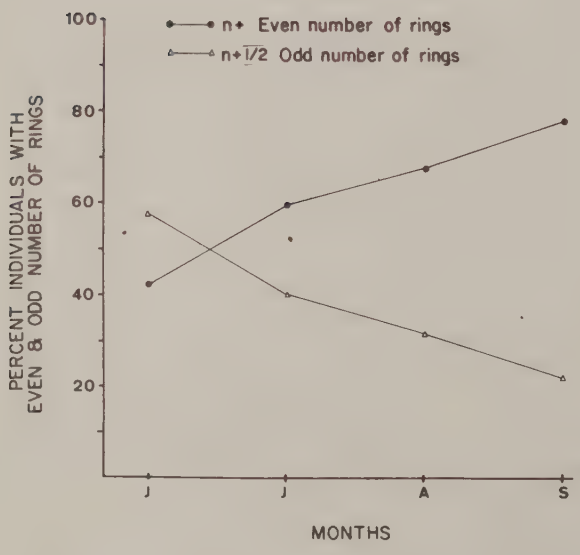

Figure 2.—Percentage of individuals collected in June-September with an even number of rings (n +) with an odd number of rings (n + ½) on dorsal spine sections.

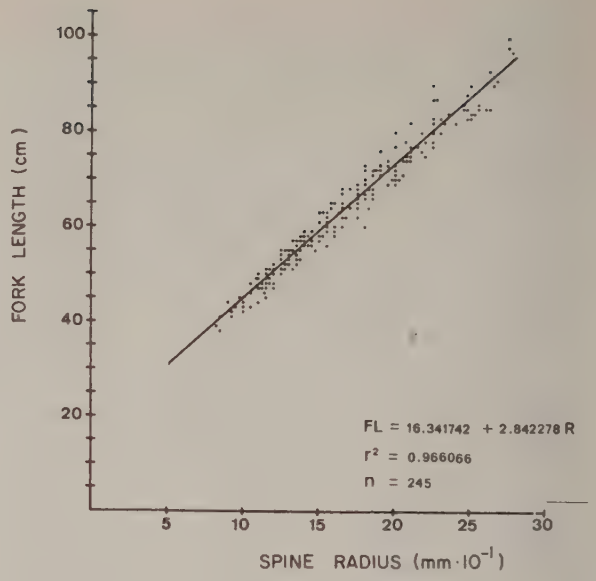

Figure 3.—Relation between spine radius (mm.10^{-1}) and fork length (cm) for 245 albacore caught in the North Atlantic, 1980-81.

Table 3.—Average radius ($\bar{x}$) of fall-winter (N_a) and spring-summer (N_b) rings (in 0.1 mm), standard deviation (SD), and the number of specimens examined (n).

Ring number	$\bar{x}$	SD	n
Ia	6.65	1.10	156
Ib	8.29	0.97	171
IIa	10.85	1.01	232
IIb	12.29	0.96	210
IIIa	14.65	1.12	154
IIIb	16.08	1.16	132
IVa	18.37	1.12	100
IVb	19.69	1.26	72
Va	21.71	1.52	41
Vb	22.74	1.59	32
VIa	24.43	1.05	13
VIb	25.53	1.11	8
VIIa	27.03	0.81	2
VIIb	27.25	—	1

resulted in the relationship: FL = 3.675784 + 0.804870 $L_{2_{DS}}$ (r^2 = 0.96, Fig. 5).

DISCUSSION

Age and growth of North Atlantic albacore have been studied since the second half of this century. Generally, previous studies have centered their analysis on young individuals that are abundant in European fisheries.

We concentrated our study on young individuals for two reasons: 1) The commercial fishery in Spain catches only immature individuals and research efforts were directed to that part of the population caught by the Spanish fleet, and 2) growth studies made on this species with spines of dorsal fins (Bard and Compeán-Jimenez 1980) are significantly different from the previous studies in two areas. For example, estimates

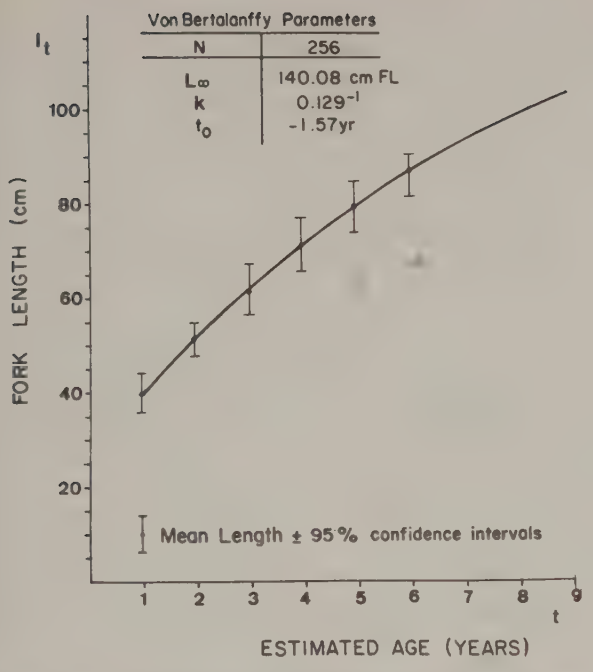

Figure 4.—von Bertalanffy growth curve estimated for young albacore of the North Atlantic based on measurements of growth rings on dorsal spine sections.

of age below 50 cm FL have not been reported, and different growth rates appear to exist between males and females after first sexual maturity (Table 1). Since mature individuals were not well represented in our samples, we could not address the question of differential growth between sexes. However, our data did allow us to investigate the size-at-age for the first year of life.

We used dorsal spines to estimate age mainly because of their ease of extraction. Interpreting the cross sections is somewhat difficult because the vascularized tissue of the central part of the spine changes in structure with the age of the fish and tends to obscure (mask) early growth rings. However, these same problems are held in common with other attempts to estimate age of oceanic pelagic fishes, particularly scombrids (Johnson 1983; Compeán-Jimenez and Bard 1983; Antoine et al. 1983; Cayré and Diouf 1983), but the overall conclusion is that spines are excellent sources of age and growth information. Also, in very young specimens, rapid early growth tends to make bands indistinct on spines. In general, the percentage of agreement between readers of cross sections is very high (96% in this study) and suggests our method of ageing is precise. The results obtained by previous studies of the growth of this species using different skeletal hardparts (Table 1) offer no clear agreement in the determination of size at 1 yr. For example, using spine cross section as a source of age information for Atlantic albacore, Bard and Compeán-Jiminez (1980) assigned an average size of 50 cm FL to fish 1+ yr old. Contrary to this, our data indicated an average length of 51 cm FL for fish 2+ yr old. Thus, a 1-yr discrepancy exists between our length at age estimates for ages 1 and 2 and those of Bard and Compeán-Jimenez (1980).

It must be noted that the smallest individuals used in Bard and Compeán-Jimenez's (1980) study were 46 cm FL. This may have hindered their observation of the first pair of rings that we usually found to be well-defined in fish $\leq$ 44 cm FL. Generally, these rings begin to become masked due to the expansion of the vascularized core of spines from specimens $\geq$ 48 cm FL. Occasionally, however, we found specimens larger than 50 cm FL showing these first pair of rings as well-defined structures, which tend to confirm our findings that 39 cm FL is the average size of albacore in their first year of life. These results agree with those obtained by Aloncle and Delaporte (1976) and Hue (1980). They estimated 40.5 cm FL and 40.8 cm FL, respectively, for the first age group of albacore that migrated to the Bay of Biscay. Therefore, we are confident that our estimate of average size for the first year of life, which is based on probable spawning dates and counts of two bands per year on dorsal spines, is accurate. However, little can be said about the accuracy of our interpretation of two bands per year on spines for older age groups, until more data are collected over the entire size range for both sexes and a directed study is conducted

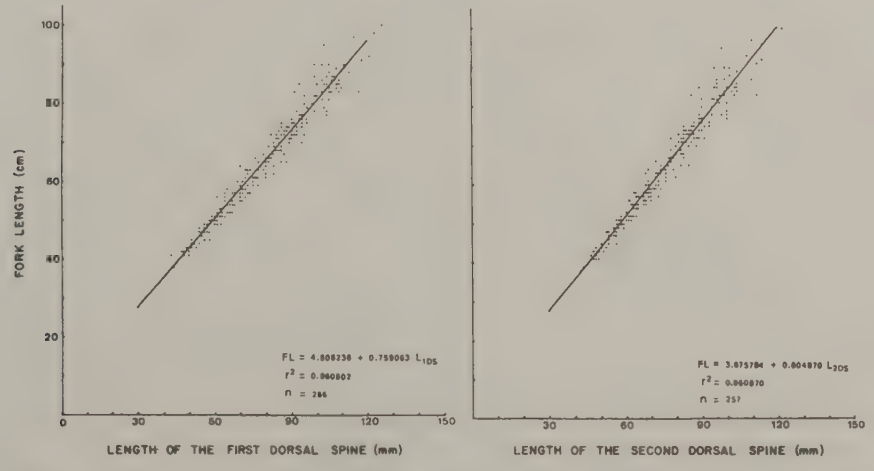

Figure 5.—Relation between fork length (cm) and length of first and second dorsal spines (mm) of young albacore (38-100 cm FL).

(such as the use of tetracycline, see Casselman 1983) for validation of our ageing method.

We observed good agreement between back-calculated fork lengths at estimated ages based on measurements of rings on spine sections and the fork lengths at estimated age predicted by the von Bertalanffy growth model (Table 4). Thus, these data indicate relatively good precision in back-calculated fork lengths at estimated age. Differentiation of growth between males and females was not conducted for this study and our use of the von Bertalanffy growth equation applies primarily to immature fish that were not sexed (up to 100 cm FL).

Since the relationships between length of the first and second dorsal spines and fork length were significant, these relationships can be used to estimate fork length when the first and second spines are available, but fish length is unknown.

Table 4.—Back-calculated fork lengths (FL back) obtained from dorsal spine ring measurements, and fork length (FL VB) rounded off to the lower cm obtained from von Bertalanffy's equation for the first 7 yr of life for eastern Atlantic albacore. Birth date was considered to be 1 July.

Estimated age	*N*	Mean FL back	FL VB
1	171	38.87	39
2	210	51.52	51
3	132	61.98	62
4	72	72.27	71
5	32	80.88	79
6	8	88.80	87
7	1	93.68	93

SUMMARY

1) Analysis of the first dorsal spine of young albacore (38-100 cm FL) indicates that our interpretation of two bands (rings) per year is accurate for estimating age for the first year of life, but we are less certain for other age categories.

2) The relationship between spine radius cross sections and fork length is linear and fits the equation:

$$FL = 16.341742 + 2.842278R \quad (r^2 = 0.97).$$

Therefore, our use of spine cross sections for back calculations is well founded.

3) The growth equation for North Atlantic albacore (38 to 100 cm FL) was found to be:

$$l_t = 140.08\,[1 - (\exp - 0.129\,(t + 1.57))].$$

4) Measurements of the first ($L_{1_{DS}}$) and second ($L_{2_{DS}}$) dorsal spines can be converted into fork length of North Atlantic albacore in the size range of 38 to 100 cm FL by:

$$FL = 4.806236 + 0.759063\,L_{1_{DS}}$$
$$FL = 3.675784 + 0.804870\,L_{2_{DS}}, \text{ respectively.}$$

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BILLFISHES

SUMMARY PAPER

Istiophorid Otoliths: Extraction, Morphology, and Possible Use as Ageing Structures

RICHARD L. RADTKE[1]

INTRODUCTION

In the past, investigators have used the progression of length modes (length-frequency analysis) to estimate age and growth of billfishes (Skillman and Yong 1976). However, this method has not been reliable because the modes in the upper range of the length-frequency distribution are often difficult or impossible to distinguish from one another due to increased overlap between year classes (Majkowski and Hampton 1983).

The method of estimating age from counts of incremental growth bands observed on skeletal hardparts is a successful technique applied to many teleosts (Bagenal 1974), but this technique has not been widely used to age billfishes. Although Jolley (1974, 1977) counted the bands on dorsal fin spines to estimate age of sailfish, *Istiophorous platypterus*, little effort has been directed towards other istiophorids. In addition, using otoliths to estimate the age of billfishes has been neglected, probably because initial reports suggested that otoliths were "minute" (undersized) and difficult to obtain for reliable age estimation (Ovchinnikov 1970). When billfish otoliths were recently examined with scanning electron microscope (SEM) techniques, however, they were found to have external and internal rhythmic depositions that could represent age information (Radtke and Dean 1981; Radtke et al. 1982). In this paper I describe a methodology for otolith extraction and morphology of otoliths from seven species of istiophorids and review evidence supporting their use as ageing structures.

METHODS AND MATERIALS

Otoliths were extracted from billfish sampled from fishing tournaments, taxidermy establishments, fish auctions, and cruises in the Pacific and Atlantic Oceans. Species examined included blue marlin, *Makaira nigricans* (N = 800 from Pacific, N = 35 from Atlantic), black marlin, *Makaira indica* (N = 5), striped marlin, *Tetrapturus audax* (N = 80), sailfish (N = 4 from Pacific, N = 100 from Atlantic), white marlin, *Tetrapturus albidus* (N = 35), longbill spearfish, *Tetrapturus pfluegeri* (N = 6), and shortbill spearfish, *Tetrapturus angustirostris* (N = 50). Total weights, sex, as well as lower jaw and/or fork to eye lengths, were recorded. Billfish sold on the market often have the bill and lower jaw removed. Thus, fork to eye lengths, in many cases, were the only accurate length measurements possible.

All otoliths were cleaned with bleach (5.25% sodium hypochlorite) and washed in 95% ethanol. The sagittae were dried for 24 h at 100°C and weighed. For external examination, all three intact otoliths were mounted on SEM observation stubs with nail polish. Nail polish is a good adhesive for attachment of otoliths to stubs, as it dries quickly and can be easily removed with acetone when various profiles of a specimen need to be examined. The mounted otoliths were gold coated and scrutinized with a SEM. Detailed descriptions of the external morphology of each of the three otoliths were consequently acquired. The nomenclature of Hecht (1978) was used to characterize the sagitta. Emphasis was given to the sagitta because it is the largest otolith and is the most common otolith used in ageing studies.

For internal structural examinations at the light microscope and SEM levels, sagittae otoliths were embedded in epoxy resin and sectioned with a low-speed rock saw on a transverse plane through the core region. The sections were polished with alumina polish (particle size 0.3 μm). For light microscopy, polished sections were mounted on glass slides using a mounting medium with the same refractive index as glass and viewed at 400 and 1,000×. For SEM observations, polished sections were mounted on stubs with epoxy resin and decalcified with 7% EDTA (disodium ethylenediaminetetraacete) at pH 7.4 (pH adjusted with NaOH) for 5 to 15 min. The specimens were then coated with gold and viewed in a SEM.

RESULTS

Extraction

Otoliths of billfish are very small and often difficult to locate. The following dissection procedures were used for extraction. Billfish were decapitated along a boundary represented by the edge of the preoperculum. A longitudinal cut was made through the midline of the head to expose the cranial cavity (Fig. 1A). Sectioning the head in this manner reveals the brain, which occupies only a small portion of the cranium. When the brain and connective membranes are displaced (Fig. 1B), the semicircular canals became visible in the lower side of the cranial pocket.

The membranous labyrinth (inner ear) of billfish, which contains the three otoliths (sagitta, asteriscus, and lapillus), was found in the cranial cavity lateral to the medulla. The sacculus, the sac that contains the sagitta, was recessed in a depression of the prootic bone in the lower posterior region of the cranial cavity. The inner ear was accommodated in a cavity ventral and posterior to the cranial chamber, with the poste-

[1]University of Hawaii at Manoa, Hawaii Institute of Marine Biology, P.O. Box 1346. Coconut Island, Kaneohe, HI 96744.

Figure 1.—(A) Exposed right section of the cranial cavity of a blue marlin; and (B) cranial cavity with the brain and connective tissues removed and the semicircular canals (SC) exposed. The lagena-sacculus chamber was recessed in a depression of the prootic bone (arrow).

rior and horizontal semicircular canals connected to the anterior semicircular canal through å foramen. It was necessary to sever the posterior semicircular canal in order to extricate the membranous labyrinth.

The lagena, which holds the asteriscus, was posterior to the sacculus, but associated in such a way that an enlarged chamber enclosed both the asteriscus and sagitta. The lagena-sacculus chamber was connected to the rest of the semicircular canals by a small membranous conduit. Special care was required for the dissection of the lagena-sacculus chamber or it would separate from the rest of the membranous labyrinth and be lost. This problem was most acute for sailfish, white marlin, striped marlin, and black marlin. Consequently, it was advantageous to first dissect the lagena-sacculus chamber from the depression in the cranial chamber in order not to lose this portion of the membranous labyrinth.

Morphology

The morphology of billfish otoliths can provide information applicable to age estimation. Sagittae otoliths from the seven species of billfish had a basic form and the prominent features common to all billfish otoliths are shown in Figure 2. The sagittae of billfish have a well-demarcated sulcus, which appeared to increase in depth with increase in fish size. Billfish sagittae lacked a collum and anterior and posterior cristae often found in other species. In the Istiophoridae, the excisural notches were very distinct and v-shaped with two lobes that folded onto each other. In most billfish otoliths, the rostrum was exaggerated with the antirostrum being approximately one-third as long and well-separated from the rostrum. The exterior of the concave portion of billfish sagittae were granular with crystalline palisade configurations. The concave surface of the rostrum was rugose and the ridges present increased in number with fish size. Scanning electron micrographs of the sagittae otoliths from seven species of billfish examined in this study are shown in Figures 3, 4, and 5.

Otoliths as Ageing Structures

External ridges on the convex surface of the rostrum were enumerated from the core region to the boundary and used for age estimation (Fig. 6A, B). These ridges were considered to be yearly in occurrence, but this assumption was not validated. In Atlantic sailfish, a fish that weighed 27.7 kg had seven rostral ridges (Fig. 6A), and a specimen that weighed 12.7 kg had three ridges. The relationship between estimated age from rostral ridge number and weight of 65 sailfish is presented in Radtke and Dean (1981) and these data suggest a rapid growth rate. In Pacific sailfish of the same species, only a few samples were collected, but a 25.9 kg fish had seven ridges while a 14.2 kg fish had four ridges. Thus, preliminary data on Pacific sailfish appear to be similar to Atlantic sailfish. In this age estimation and others for different species, the number of rostral ridges

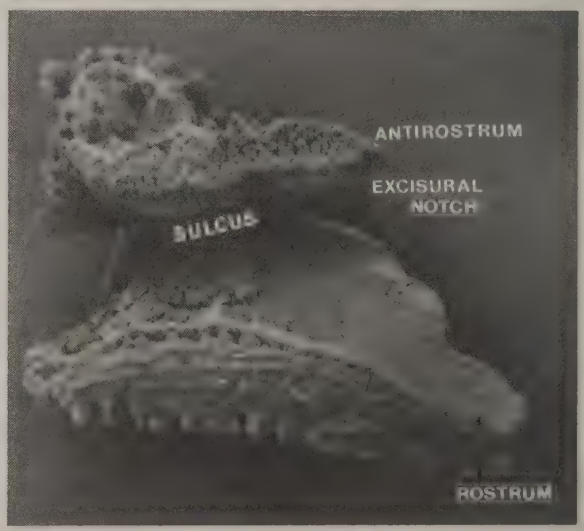

Figure 2.—Medial view of an istiophorid sagitta otolith (7×). Major morphological features include: Antirostrum, excisural notch, sulcus, and rostrum.

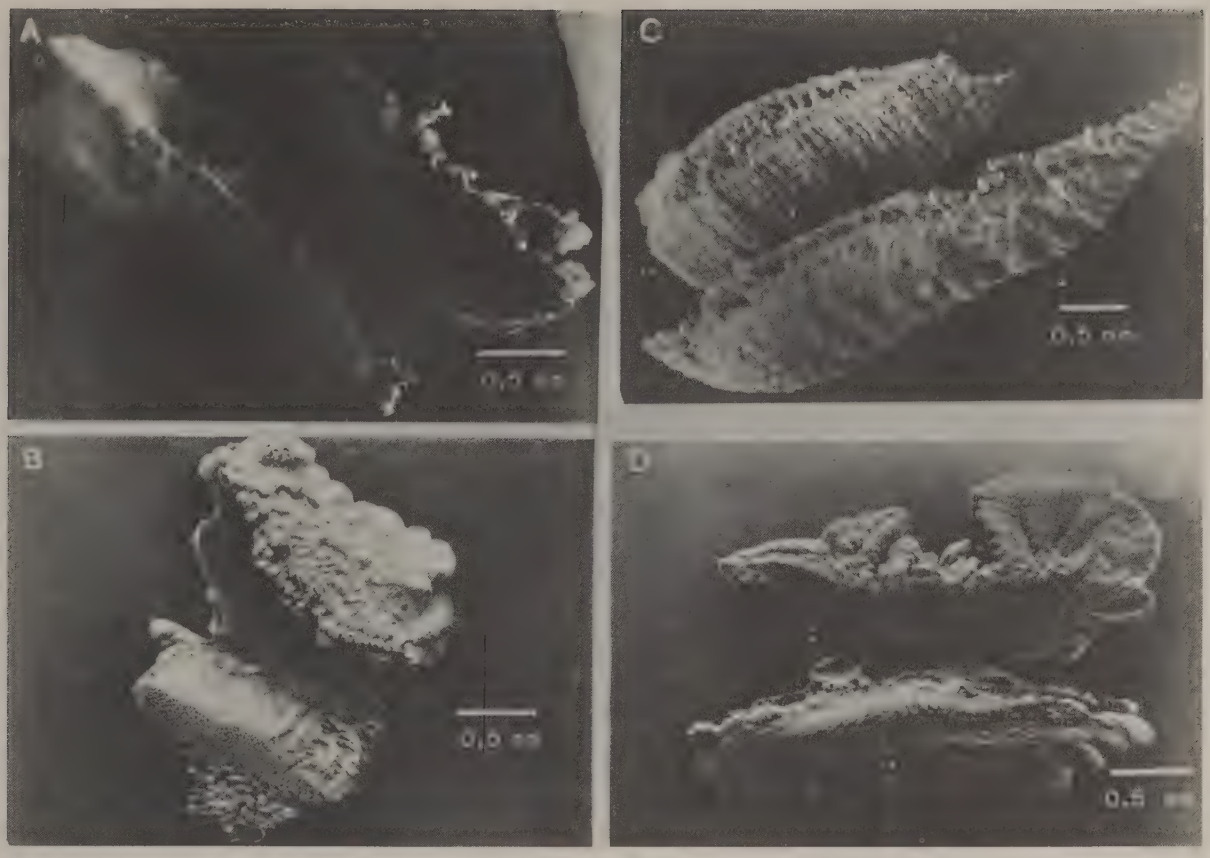

Figure 3.—Scanning electron micrographs of the sagittae otoliths from: (A) 89 kg Atlantic blue marlin; (B) 262.2 kg Pacific blue marlin; (C) 28 kg Atlantic sailfish; and (D) 27 kg Pacific sailfish.

was found to have a stronger relationship with weight than with length.

In Atlantic blue marlin, a significant correlation ($r = 0.96$) was found between the size of the fish and the number of ridges on the convex surface of the rostral lobe (Radtke et al. 1982). Supportive evidence for annual ridge deposition in blue marlin was furnished from an available tagged and recaptured specimen (Mather et al. 1974), which at initial capture weighed approximately 90 kg. When recaptured after 30 mo at liberty, it weighed 163 kg (eviscerated). When the data from the tagged fish was plotted on the age estimate regression for Atlantic blue marlin, the fit to the line was within the 95% confidence interval. In the Pacific, cursory examination of otoliths from 14 blue marlin also indicated increases in number of rostral ridges with an increase in weight and thus show potential for estimating age (Table 1). In addition, these preliminary data revealed interesting insights into the life history of Pacific blue marlin. Males appear to grow more slowly than females and may not attain the maximum age or size of females. Examination of otoliths from a much larger sample size ($N = 800$) may provide additional insight into these trends.

Table 1.—Sagitta otolith weight (mg) and counts of ridges on the rostral lobe of otoliths (estimated age) of 14 Pacific blue marlin.

Eye to fork length (cm)	Total weight (kg)	Sex	Otolith weight (mg)	Estimated age ridge count
294	337.72	F	4.49	17
270	287.27	F	4.81	16
268	227.73	F	6.28	15
243	229.55	F	6.66	13
248	210.00	F	4.99	13
217	154.09	F	2.79	8
241	135.91	F	3.29	8
191	97.27	M	3.34	12
189	114.55	M	1.48	11
181	82.27	M	5.32	9
177	71.36	M	.91	8
176	68.18	M	1.41	8
177	68.64	M	2.67	7
165	52.27	M	2.22	6

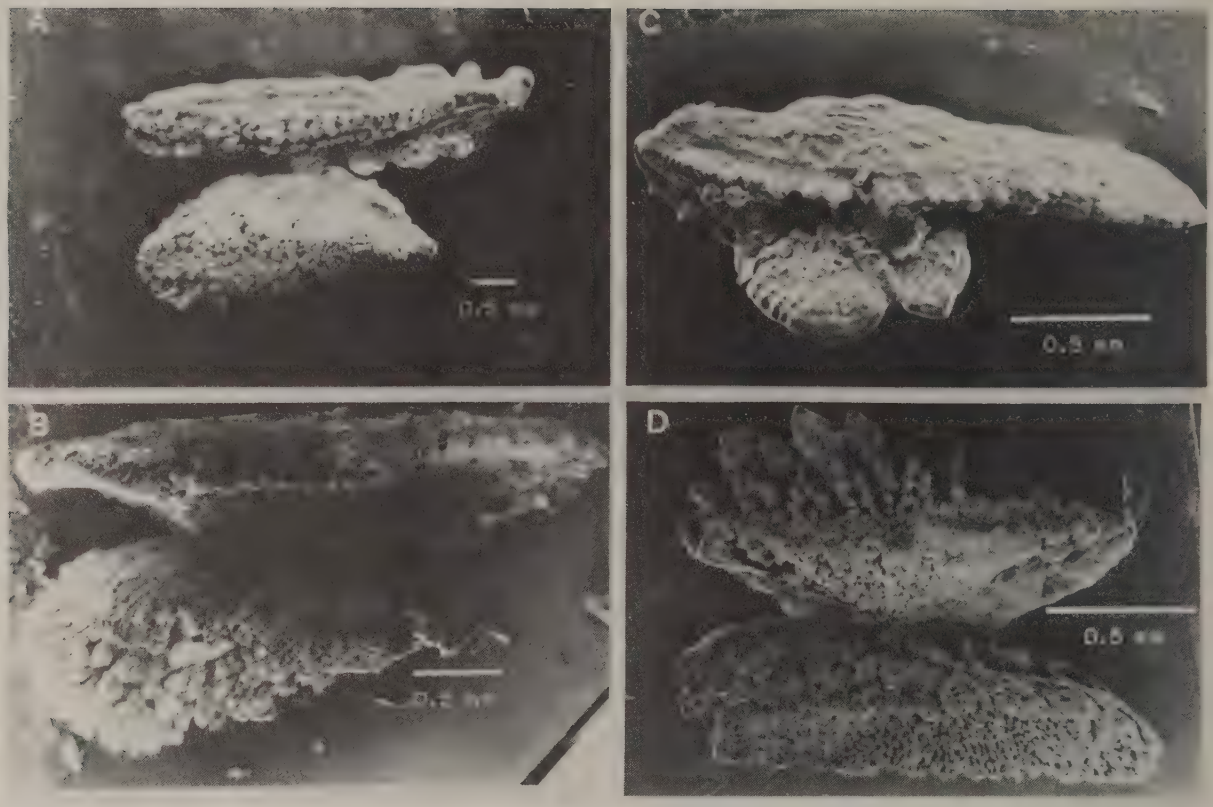

Figure 4.—Scanning electron micrographs of the sagittae otoliths from: (A) 62 kg striped marlin; (B) 70 kg white marlin; (C) 15 kg longbill spearfish; and (D) 10 kg shortbill spearfish.

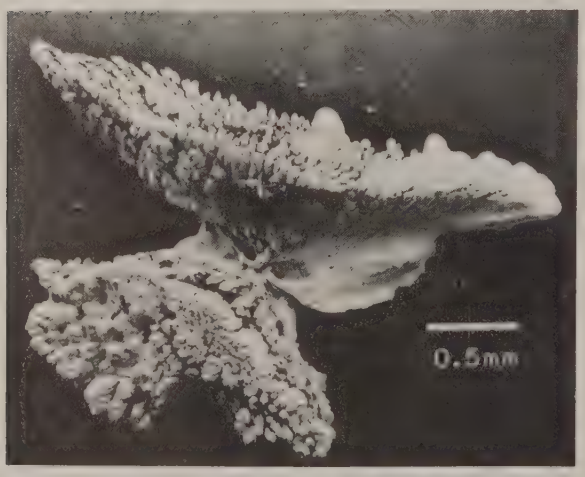

Figure 5.—Scanning electron micrograph of the sagitta otolith from a 116 kg black marlin. Bar = 0.5 mm.

In addition to sailfish and blue marlin, the range in age estimates based on counts of rostral ridges for the five other species of billfish showed a maximum age for black marlin of 18 yr and a minimum age of 1 yr for shortbill spearfish (Table 2). Data collected to date demonstrate that ridges can be found on the rostral lobes of all istiophorids (Fig. 6A, B) and they could be used for age estimation once they are validated. Examining internal structures of billfish otoliths by SEM and light micro-

Table 2.—Sample size (N), range in weight, and range of counts of ridges on rostral lobe of sagittae otoliths used for estimating age of five species of istiophorids.

Species	N	Weight range (kg)	Ridge count range
Striped marlin	80	18-62	3-6
White marlin	35	10-32	3-5
Black marlin	5	67-284	6-18
Longbill spearfish	6	8-28	2-5
Shortbill spearfish	50	4-21	1-6

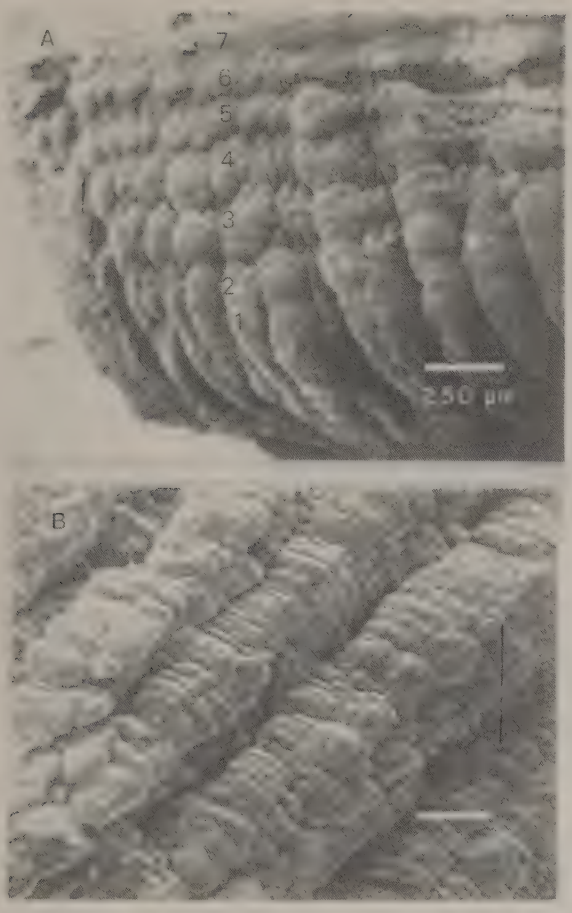

Figure 6. – (A) Rostral lobe of the left saggita of a 27.7 kg sailfish (34 ×). Numbers indicate ridges. Bar = 250μm. Adopted from Radtke and Dean (1981); and (B) ridges on the rostral lobe of a sagitta from a 220 kg Pacific blue marlin. Bar = 200μm.

scopy revealed laminations which indicated rhythmic incremental otolith growth and progressive deposition (Fig. 7). These examinations suggested that the external ridges were extensions of internal rhythmic otolith growth and could be governed by an annual or a seasonal cycle. This assumption has been validated for other teleosts (Bagenal 1974) and could also apply to billfish.

The sagittae otoliths of billfish displayed a wide range of visual morphological patterns and weights (Figs. 3, 4, 5) which showed a priori intraspecific differences. Some of the intraspecific variations were in rostral length, while other variations were in general otolith shape that has not yet been quantified. However, size of billfish and weight of otoliths were not closely related (Table 1). For example, a female blue marlin weighing 337.7 kg had an otolith weight of 4.49 mg, while another female weighing only 287.2 kg had an otolith weight of 4.81 mg (Table 1). In most fish species, the size of the sagitta increases with the size of the fish. Neither estimated age, sex, nor fish size appeared to have any relationship to sagitta size, but a relationship may exist which would become evident upon the analysis of a larger sample size. The deviation from normal expectancies of teleost otolith shape and size may bring new insights into the understanding of otolith use or may negate the use of billfish otoliths for age estimation.

The asteriscus and lapillus were much smaller than the sagitta. They both showed external and internal features that appeared to have progressive deposition. The asteriscus was very fragile and was often broken upon dissection. The lapillus was much heavier than the asteriscus and its growth features supported the sagittae ridge counts. The lapillus also showed incremental layers when viewed under transmitted light.

DISCUSSION

Billfish otoliths were conspicuously smaller in relation to the size of otoliths from other fish species (Fig. 8). This relationship may explain why they have been overlooked in the past for estimating age of istiophorids. Still, with the proper techniques it is practicable to examine these minute otoliths for in-

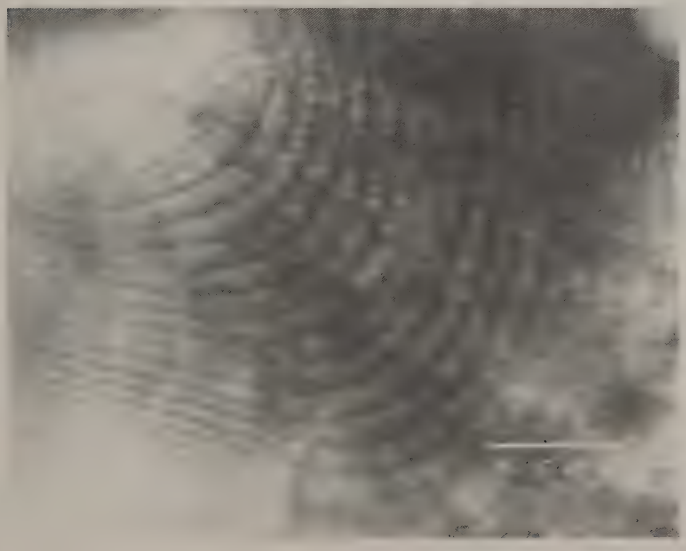

Figure 7. – Light micrograph of a sectioned sagitta from a 54 kg striped marlin showing internal growth increments. Bar = 40μm.

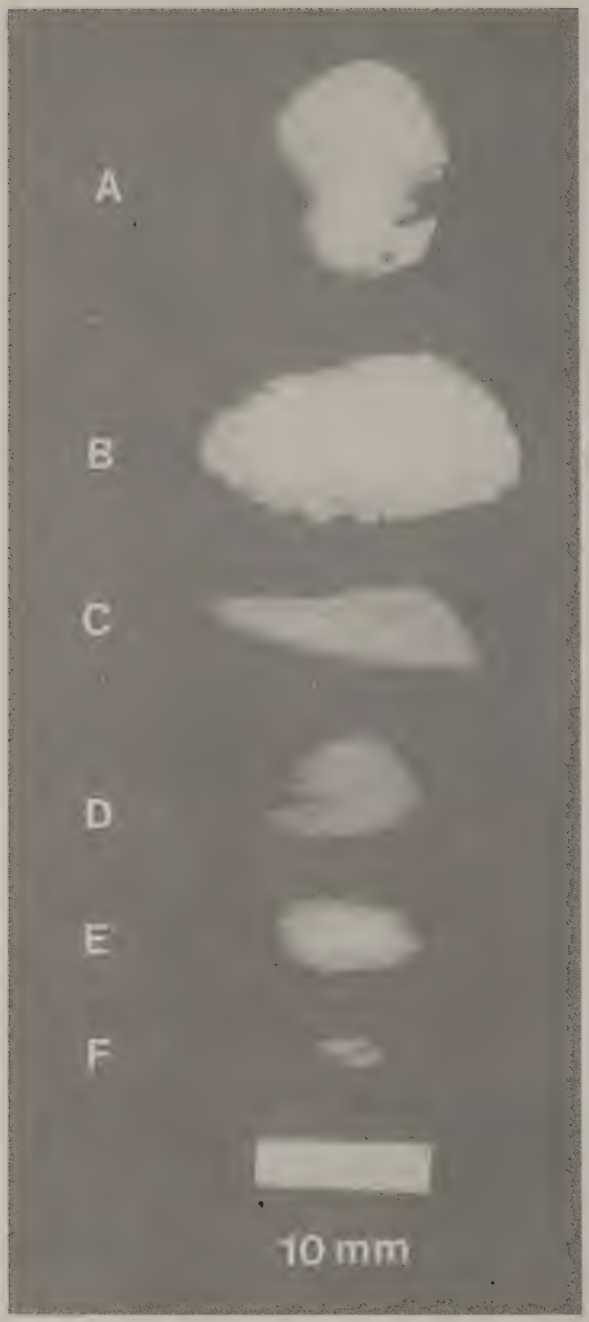

Figure 8.—Size relationship of sagittae otoliths of selected fish species: (A) 1 kg Atlantic croaker, *Micropogon undulatus*; (B) 3 kg Atlantic cod, *Gadus morhua*; (C) 85 kg yellowfin tuna, *Thunnus albacares*; (D) 2.7 kg gray snapper, *Lutjanus griseus*; (E) 300 gm sailfin flyingfish, *Parexocoetus brachypterus*; and (F) 248 kg blue marlin. Bar = 10 mm.

cremental growth patterns that may be daily and yearly in occurrence and that have been documented as reliable sources of age information for other teleosts (Bagenal 1974).

Otoliths of teleosts typically consist of needle-shaped crystals of calcium carbonate that radiate in three dimensions from a core region contained in an organic network (Williams and Bedford 1974). Otolith growth occurs when new material is deposited on the outer surfaces. This material usually takes two forms (aragonite and protein), which are deposited in an alternating sequence forming concentric rings. Typical variations in the internal deposition of protein and aragonite crystals, which have been interpreted as annual events in other teleosts (Williams and Bedford 1974), were also evident from the formation of growth increments viewed on sections of billfish otoliths. Moreover, the deposition of external rostral ridges seen on billfish otoliths could therefore conceivably be a product of such variation in crystal formation extending to the outer surface of these otoliths. The use of such criteria could provide the basis for making estimates of age using istiophorid otoliths.

Few age estimates exist for billfish. Jolley (1974, 1977) was one of the first to utilize hard structures (dorsal spines) for estimating age, and his data on sailfish were very similar to the data gathered on sailfish otoliths by Radtke and Dean (1981). However, sailfish otoliths had a lower rejection rate than spines ($< 1\%$) and in this instance made it feasible to estimate age of larger specimens. This relationship tends to lend credibility to the use of otoliths as records of age for istiophorids. Wilson and Dean (1983) and Radtke and Hurley (1983) also used otoliths for age estimation of billfish, while Hedgepeth and Jolley (1983) and Berkeley and Houde (1983) used spines for age estimation. While these studies and my study demonstrate that age estimation of billfish is possible and provides a genesis for future research, true age validation has not been accomplished (see Brothers 1983).

The internal structural features of billfish otoliths may represent incremental growth as has been shown for other teleost species (Bagenal 1974). The small internal increments of billfish otoliths were similar to the daily increments observed in the sagittae of other fish species (Brothers et al. 1976; Radtke and Dean 1981). Yearly and daily increments appear to be common to most teleost fish and it is reasonable to expect that billfish otoliths also function as sources of age information. The external and internal structure of billfish otoliths may be a permanent calendar of the life history of the fish, but further investigation is necessary to extract all the information contained within the otoliths.

The morphology of billfish otoliths interjects some interesting questions. The morphological shapes and weights of billfish otoliths have definite inter- and intra-specific variations that cannot be explained. Speculation on these variations could point to some future areas of research which may shed some light on these issues. Fish otoliths have been shown to denote population structure (Postuma 1974), and the wide differences in shape and size of billfish otoliths could be population or environmentally related. However, the lack of a strong relationship between size of billfish and size of their otoliths may negate the use of otoliths for back calculation of previous growth history (Smith 1983). Wilson and Dean (1983) also demonstrated the lack of a strong relationship between size of broadbill swordfish, *Xiphias gladius*, and size of their otoliths.

In conclusion, the present study documented the methods necessary to find and view the minute otoliths of billfish. Billfish otoliths do have structures which were analogous to structures used in other fish species for age estimation. SEM examination of the morphological and structural features of billfish otoliths has the potential to provide information on age and, to a lesser extent, growth of these large oceanic pelagic fish.

Otoliths of billfish may not be a remedy for all the questions concerning billfish biology and ecology, but they may provide insight into age and growth aspects of billfish life history.

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[2]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Age and Growth of Sailfish, *Istiophorus platypterus*, Using Cross Sections from the Fourth Dorsal Fin Spine

MARION Y. HEDGEPETH[1] and JOHN W. JOLLEY, JR.[2]

ABSTRACT

Cross sections from fin spines of 1,071 sailfish, *Istiophorus platypterus*, obtained from the sport fishery in southeast Florida (1970-80), were examined to estimate age and growth rates. Growth bands on 53% (569) of the spines were legible and age estimates ranged from 1 to 7 yr. Maximum age may exceed 7 yr since we were not able to age the largest sailfish. The most abundant age groups were 3 and 4. Female sailfish were slightly larger than males and may live longer. No males were observed exceeding estimated age 6. Generally, good agreement was obtained between observed, back-calculated, and theoretical growth. The von Bertalanffy growth equations for length of males and females were:

$$l_t = 147\ [1\text{-exp}(-0.3014)(t+1.959)]$$

$$l_t = 183\ [1\text{-exp}(-0.1586)(t+3.312)],\ \text{respectively.}$$

Sailfish were found to be a relatively fast-growing, oceanic pelagic species, although we estimated annual growth rates to be slower and more gradual that previously reported in the literature. Estimates of instantaneous total mortality (Z) ranged from 1.14 to 1.90 for males and from 0.82 to 1.15 for females.

INTRODUCTION

Age and growth of sailfish, *Istiophorus platypterus*, were estimated first by deSylva (1957) using length frequency analysis (Petersen method). He noted three modes (year groups) in the frequency distribution, and thus concluded that sailfish were a very fast-growing, short-lived species. Variations in sailfish length-weight relationships by sex were later reported by Williams (1970), Maksimov (1971), Nakamura and Rivas,[3] Wares and Sakagawa (1974), and Jolley (1974, 1977). These studies suggested differences in growth rates or longevity between males and females. Estimates of age and theoretical growth of sailfish have been reported by Jolley (1974, 1977), Radtke and Dean (1981), and Farber.[4] Jolley (1974) explored the use of several hardparts, including vertebrae, for age estimation. Dorsal fin spines were found to be the most promising structures (particularly the fourth dorsal spine). Jolley (1974) reported a significant relationship between trunk length and spine radius ($r = 0.90$), which justified use of spines for back calculations of previous growth history. He also theorized that the maximum age of sailfish may be 9 or 10 yr; however, he was unable to verify this due to the illegibility of bands on spine sections from large sailfish. Using scanning electron microscopy, Radtke and Dean (1981) examined the morphological features of 65 sailfish otoliths and were able to use 98% of the otoliths to estimate age. The estimated age of their largest specimen was 7 yr, which concurred with Jolley (1977). Farber (footnote 4) examined historical release-recapture data of tagged sailfish and other billfish to determine growth rates, mortality rates, and migration patterns. He indicated a maximum age of approximately 6 yr and an asymptotic size achieved by age 3. Thus, questions concerning maximum longevity and growth rates of sailfish remain unresolved.

In our study, we used cross sections from the fourth dorsal fin spine to determine the age of sailfish and to obtain estimates of back-calculated and theoretical growth, and mortality.

MATERIALS AND METHODS

Dorsal fin spines were taken from 1,071 sailfish captured primarily by the sport fishery off southeast Florida between 1970 and 1980. One hundred forty-nine of the spine sections were utilized by Jolley (1977) in his preliminary analysis of sailfish age. Measurements of trunk length in centimeters (TKL, the length between the posterior edge of the orbit to the origin of the caudal keels) and total weight in kilograms were taken from each specimen. Sex was determined macroscopically. Dorsal fin spines were cut and prepared according to the methods of Jolley (1977). Only sections from the fourth dorsal fin spine were utilized in the back calculation of growth analysis. Sections from each spine were stored dry, placed in glycerine, and read under a binocular microscope (10×) equipped with reflected light and a dark background. Broad opaque bands and narrow translucent bands alternated outward from the central core (see Glossary). Translucent bands that continued around the entire circumference of the spine were considered annuli (see Glossary) and the total number of these bands were recorded in order to assign ages to each specimen. We assumed

[1]Florida Department of Natural Resources, Marine Research Laboratory, 100 Eighth Avenue SE., St. Petersburg, FL 33701.

[2]Shearson/American Express Inc., 75 N. Federal Hwy., Suite 101, Delray Beach, FL 33444.

[3]Nakamura, E. L., and L. R. Rivas. 1972. Big game fishing in the northeastern Gulf of Mexico during 1971. Unpubl. mimeo. Southeast Fisheries Center Panama City Laboratory, National Marine Fisheries Service, NOAA, 3500 Delwood Beach Road, Panama City, FL 32407.

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that the distance between translucent bands represents 1 yr growth based on previous work (Jolley 1974, 1977; Radtke and Dean 1981), but this assumption remains, in part, unvalidated. The size of each growth band was measured from the center of the core through the middle of the right hemisphere of the section to the outer edge of each translucent zone (annulus). Three readings of each spine by two readers were made independently. If agreement between readers could not be reached, these spines were not used in the analysis.

The relationship between TKL and spine radius was determined with regression analysis. All statistical inferences were based on a significance level of $\alpha = 0.05$. Back calculations of length-at-estimated age were obtained from the following equation (Tesch 1971; Ricker 1975):

$$L_n - c = (S_n/S)\,(L - c) \qquad (1)$$

where L_n = the length of the fish when the annulus (n) formed,
L = the length of the fish at the time of capture,
S_n = a measure of the size of each annulus,
S = the radius of the right hemisphere of the spine,
c = a correction factor (y-intercept of the regression fish trunk length vs. radius of the right hemisphere of the spine).

Estimates of theoretical growth in length of sailfish were obtained by fitting the spine measurement data to the von Bertalanffy growth equation following the Beverton method in Ricker (1975:225). Theoretical growth-in-weight was obtained by converting length to weight (Gulland 1969:39) using the length-weight relationship of Jolley (1974).

Instantaneous total mortality rates (Z) were estimated by four methods: 1) Heinke (Everhart and Youngs 1981), 2) Jackson (Everhart and Youngs 1981), 3) Chapman and Robson (Everhart and Youngs 1981), and 4) Beverton and Holt (1957). Frequency of observed age groups was used to obtain estimates of Z. Age group 4 was considered as the first fully recruited year class of sailfish to the recreational fisheries off the coast of southeast Florida (Jolley 1977).

RESULTS

Of the 1,071 sailfish spines examined to estimate age, 569 of the cross sections were legible (259 were males and 310 were females). There was a significant linear relationship between fish trunk length and spine radius ($r = 0.77$). Estimated age groups 3 and 4 were the most abundant year classes (Fig. 1). The mean age group of both sexes was 4. Maximum age may exceed 7 yr, since spine cross sections from large sailfish (> 155 cm TKL) were not legible due to the accumulation of oil in the core of spines or masking of growth bands because of enlargement of the core. The greatest variation in length of sailfish occurred in estimated age group 2. Females grew larger than males, and were more variable in length and weight.

Mean observed, back-calculated, and theoretical growth were compared in Table 1 and Figures 2 and 3. By estimated age 1, males obtained a mean back-calculated trunk length of 90 cm and a mean back-calculated weight of 5.7 kg. Estimated age 6 males averaged 135 cm TKL and 19.3 kg; however, no males exceeded 6 yr of age. Estimated age 1 females had a mean back-calculated trunk length of 91.9 cm and a mean back-calculated weight of 9.1 kg. By estimated age 7, females

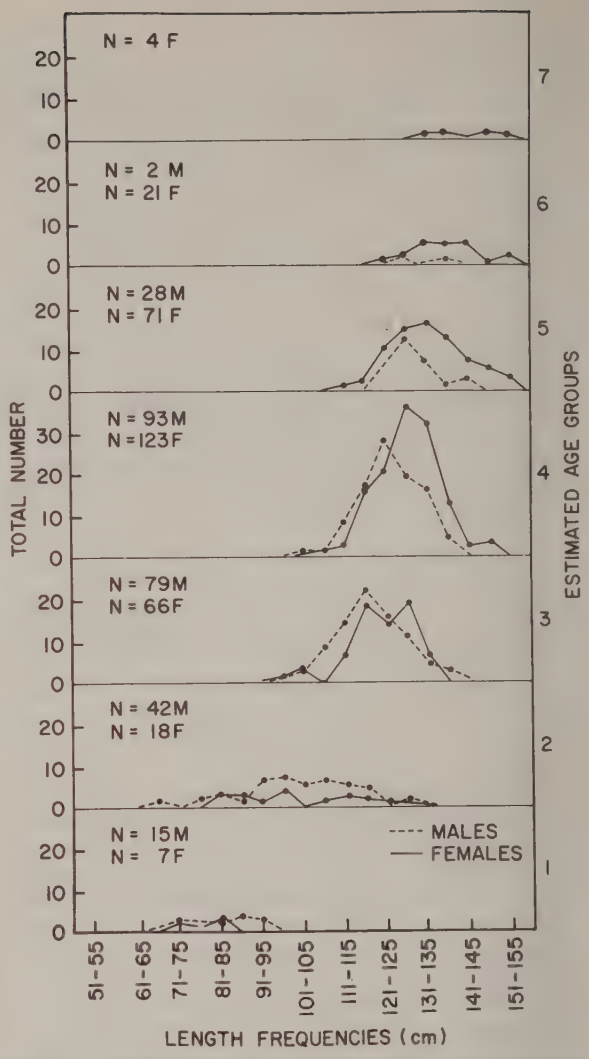

Figure 1.—Length frequencies of male and female sailfish by estimated age groups from the northwestern Atlantic Ocean.

averaged 151.4 cm and 34.6 kg. Growth curves for observed, back-calculated, and theoretical data agreed more closely for male sailfish (Fig. 2) than for female sailfish (Fig. 3). Mean back-calculated trunk lengths (Table 2) illustrated that both sexes grew at approximately the same rate (in length) during their first year; however, females grew faster than males in length after the first year of life.

The von Bertalanffy equations for the theoretical growth in length (l_t) and weight (w_t) for male sailfish were:

$$l_t = 147\,[1 - \exp(-0.3014)(t + 1.959)]$$

$$w_t = 28.8\,[1 - \exp(=0.3014)(t + 1.959)]^{3.342}, \text{ respectively.}$$

The von Bertalanffy equations for the theoretical growth in length (l_t) and weight (w_t) for female sailfish were:

$$l_t = 183\,[1 - \exp(-0.1586)(t + 3.212)]$$

$$w_t = 54.1\,[1 - \exp(-0.1586)(t + 3.212)]^{2.950}, \text{ respectively.}$$

Table 1.—Summary of observed, back-calculated, and theoretical growth in length (trunk length, TKL, and total length, TL) and total weight of sailfish from the present study and other studies in the North Atlantic Ocean and the East China Sea.

		Present study						DeSylva[1] (1957)		Koto and Kodama[1] (1962)		Farber[2,3] (1981)	
		Observed		Back-calculated		Theoretical							
	Estimated age	male	female	male	female	male	female	(Sexes combined)		(Sexes combined)		(Sexes combined)	
		TKL		TKL		TKL		TKL	TL	TKL	TL	TKL	TL
Length (TKL and TL, cm)	1	80.6	79.1	90.0	91.9	86.7	89.2	108.8	182.9	92.2	157.5	89.7	153.7
	2	100.4	100.8	102.4	104.5	102.4	102.9	130.4	215.9	110.2	185.0	119.7	199.6
	3	118.6	122.0	113.2	114.9	114.0	114.7	142.0	233.7	121.6	202.5	128.7	213.3
	4	124.2	128.6	120.2	123.8	122.6	124.7					131.3	217.4
	5	129.9	133.5	127.9	128.2	128.9	133.2					132.1	218.6
	6	132.3	139.7	135.1	136.7	133.6	140.5					132.4	218.9
	7		144.4		151.4		146.8					132.4	219.1
Total weight (kg)	1	3.9	3.2	5.7	9.1	4.9	6.5	9.5					
	2	8.5	8.5	9.6	13.1	8.6	9.9	19.5				2.0	
	3	14.6	15.8	12.3	17.7	12.3	13.6	28.6				7.4	
	4	17.2	19.0	15.1	21.8	15.7	17.4					14.8	
	5	18.7	20.9	18.1	25.0	18.6	21.2					22.0	
	6	18.9	26.9	19.3	29.2	20.9	24.8					26.8	
	7		32.4		34.6		28.2					30.5	
												33.0	

[1]Modes of total length to trunk length conversions, and mean total weights derived from length frequency analysis (sexes combined).
[2]Total length to trunk length conversions from Jolley (1977) and total weights derived from tag returns (sexes combined).
[3]See text footnote 4.

Instantaneous total mortality estimates ranged from 1.00 to 1.35 for all fish combined (Table 3). All four methods of estimating mortality gave higher values of Z for males (1.41-1.90) than for females (0.82-1.15). These estimates may be high due to our inability to age older fish, which would result in underestimating abundance of older age groups.

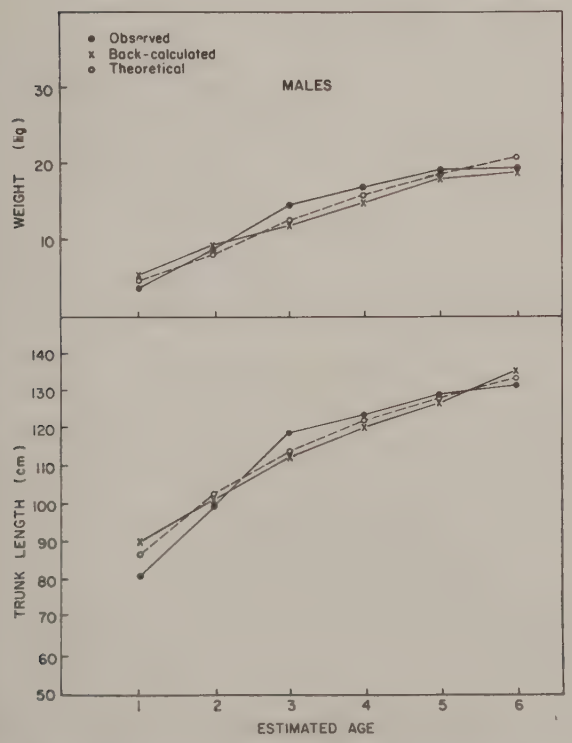

Figure 2.—Mean observed, back-calculated, and theoretical growth of 259 male sailfish from the northwestern Atlantic Ocean.

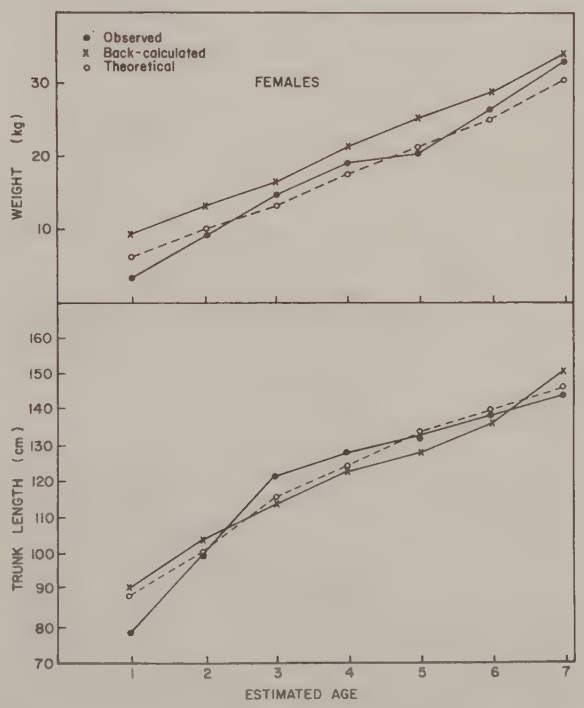

Figure 3.—Mean observed, back-calculated, and theoretical growth of 310 female sailfish from the northwestern Atlantic Ocean.

Table 2.—Mean back-calculated trunk lengths at estimated age for sailfish caught off southeastern Florida, 1970-80.

Age group	N	Back-calculated trunk length (cm) at estimated age						
		1	2	3	4	5	6	7
		Combined data						
1	21	77.2						
2	58	87.0	97.8					
3	141	92.7	106.5	116.2				
4	214	92.6	103.9	114.7	123.4			
5	98	92.0	101.7	111.2	120.4	128.4		
6	24	89.5	101.0	112.4	121.8	128.8	135.3	
7	5	87.4	100.8	115.3	126.8	133.9	139.2	144.5
Total	561							
		Male						
1	14	77.3						
2	40	86.8	97.1					
3	75	92.0	106.7	115.2				
4	93	92.3	102.7	112.8	121.1			
5	28	91.2	101.0	111.5	120.0	127.8		
6	2	—	93.8	105.0	116.8	124.5	130.2	
Total	252							
		Female						
1	7	76.9						
2	18	87.3	99.4					
3	66	93.4	106.2	117.4				
4	120	92.8	104.8	116.2	125.2			
5	68	92.2	102.0	111.1	120.5	128.5		
6	22	89.5	101.7	113.1	122.2	129.2	135.8	
7	5	87.4	100.8	115.3	126.8	133.9	139.2	144.5
Total	306							

Table 3.—Instantaneous total mortality (Z) estimates for sailfish caught off southeastern Florida, 1970-80.

Method[1]	Male	Female	Combined
Heinke	1.41	0.82	1.00
Jackson	1.41	0.82	1.00
Chapman and Robson	1.57	1.01	1.15
Beverton and Holt	1.90	1.15	1.35

[1]Methods of calculating total mortality (Z) given in Everhart and Youngs (1981).

DISCUSSION

Sailfish age distributions and sizes at age closely paralleled those proposed by Jolley (1977). However, the relationship between fish trunk length and spine radius in this study ($r = 0.77$) was not as high as reported by Jolley ($r = 0.99$, 1977). This may be due to the nonsymmetrical growth of some spines and/or differences in sample sizes between studies.

Mean observed, back-calculated, and theoretical lengths and weights-at-estimated age appeared to be realistic and relatively consistent for the sailfish we examined. Growth in weight was very rapid (exponential) during the first 3 yr of life, but appeared to become asymptotic thereafter. Thus, the von Bertalanaffy growth model more accurately reflected growth in later years. Other growth models were not used and we did not intend to imply that the von Bertalanaffy model was the most appropriate. This model was used for the convenience of making comparisons with other studies. Sailfish seem to have a particularly rapid growth rate when compared with other billfishes (Berkeley and Houde 1983). However, growth rates in this study were slower than those suggested for sailfish by deSylva (1957), who estimated an annual growth rate of approximately 130 cm TKL in the first 2 yr; we estimated about 5 yr to attain a length of 150 cm TKL in this study. Growth data analyzed by Farber (footnote 4) suggested that an asymptotic size is reached by age 3, followed by some minor growth after this period; whereas, we found a more gradual rate of growth, as Jolley (1977) initially proposed in his comparison of age-weight relationships. Radtke and Dean (1981) reported good agreement between their ageing technique using otoliths and spine analysis by Jolley (1977) and these data also tend to support a more moderate rate of growth. However, modes in length of sailfish from the East China Sea as observed by Koto and Kodama (1962) indicated that sailfish from this region may grow somewhat faster than sailfish in the western Atlantic Ocean.

As evident from reports of several authors (Antoine et al. 1983; Berkeley and Houde 1983; Cayré and Diouf 1983; Compeán-Jimenez and Bard 1983; González-Garcés and Fariña-Perez 1983; Johnson 1983), many oceanic pelagic fishes exhibit doubling or tripling of growth bands on spines. Sailfish were no exception. We attributed multiple banding to the actual splitting of the annulus which was observed ventral and/or dorsal to the core of the spine. The cause of this multiple banding was unclear; however, if one annulus was double or triple, generally, other annuli formed thereafter exhibited the same trend. These multiple bands were observed in all age groups.

Our combined estimates of instantaneous total mortality, which ranged from 1.00 to 1.35, were similar to those values reported by Farber (footnote 4; $Z = 0.90$) and Buchanan et al.[5] ($Z = 1.112$). Farber's mortality estimates, which were based on release and recapture data, ranged from 0.405 to 2.197. Slight differences between our current estimates of Z and those of other studies were probably the result of variations in proposed growth rates and age estimations (including the inability to age older fish) and methods of analysis. To further substantiate estimates of age and growth rates, studies on validation using mark-recapture or tetracycline marking should be incorporated into future research.

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Age Determination of Broadbill Swordfish, *Xiphias gladius*, from the Straits of Florida, Using Anal Fin Spine Sections

STEVEN A. BERKELEY[1] and EDWARD D. HOUDE[2]

ABSTRACT

A total of 439 swordfish, *Xiphias gladius*, from the Florida Straits, collected between 1978 and 1980, were aged from bands present on thin sections of the second element of the anal fin. Eighty-seven percent of all swordfish spines were readable, although percentage readibility declined in the largest size classes of males. While there is considerable individual variation in time of band formation, most bands apparently are laid down in winter. There is a significant linear relationship ($r = 0.94$) between fin spine radius and lower jaw fork length (LJFL). Bands assumed to be annual events were counted under a dissecting scope and used to back calculate lengths at estimated age, which were used to fit the von Bertalanffy growth model. The differences in size and growth rates of males and females are shown by the resulting parameter estimates: $L_\infty = 217.4$ cm LJFL, males; $L_\infty = 340.0$ cm LJFL, females; $k = 0.19$, males; $k = 0.09$, females; $t_0 = -2.04$ yr, males; $t_0 = -2.59$ yr, females. Although older fish exist in the population, the oldest fish in our sample was age 11; 61% were < 4 yr old. Despite some remaining problems in reading fin spines, three advantages in using spines in an age and growth analysis of swordfish are: 1) They are easily obtained, 2) they present few handling and storage problems, and 3) they are inexpensive to process and read.

INTRODUCTION

Accurate determination of fish ages is needed to estimate growth and mortality rates and to fit yield models. For swordfish, *Xiphias gladius*, attempts at ageing have been made by using several techniques, but there are no definitive studies on the swordfish's age and growth. Until recently, most preliminary size at age or growth estimates have been based on modal analysis of size frequency distributions (Yabe et al. 1959; Kume and Joseph 1969; Beckett 1974; Ovchinnikov et al. 1980). Artüz (1963) sectioned dorsal fin spines of swordfish from the Sea of Marmara and found marks that he believed were annual events, but he did not present age or growth estimates. Tag returns have provided some information on swordfish growth rates, but the small number of tag returns and inaccurate estimates of size at tagging have limited the information available from this method.

In 1978, the University of Miami and the Florida Department of Natural Resources began an investigation of the fishery and biology of broadbill swordfish from the Florida Straits, which included an age and growth study. The work of Artüz (1963) and the successful ageing of sailfish, *Istiophorus platypterus*, by Jolley (1974, 1977) prompted us to examine fin spines as a means of ageing swordfish. Recently, Beamish (1981) reviewed the literature on fin rays and their successful use in ageing fishes, including large pelagic species such as albacore, *Thunnus alalunga*.

In this paper we describe a method to age swordfish using the second anal fin spine as the source of age information to estimate ages, to back-calculate length at estimated age, and to fit the von Bertalanffy growth model.

METHODS

Swordfish were sampled from both recreational fishing tournaments and commercial longline catches from the Florida Straits. Various hardparts were collected initially to determine their potential use for ageing. These included dorsal, anal, and pectoral fin spines, caudal vertebrae, and otoliths. Anal fin spines were found to be the most suitable structures, having clear marks, a well-defined focus, and a small inner matrix area.

Anal fins were collected aboard fishing vessels or at dockside along with lower jaw fork length (LJFL), round or dressed weight, sex, and other biological data. Excised fins were labeled, placed in plastic bags, and frozen. Later, fins were thawed and individual elements separated and cleaned of skin and tissue. The second spine, although not the longest, was determined to be best for ageing because it had the smallest matrix and largest diameter. This spine was sectioned above the base, using a razor saw, at the point where the spine flares (condyle), and two or three cross sections (about 1 mm thick) were cut distally. Sections were stored in vials containing 5% Formalin.[3] Because each spine consists of two halves joined along the midline, the halves of each section were separated with a scalpel before reading. Sections were read twice by a single reader under a dissecting microscope at either 6×, 12×, or 25× magnification, depending on spine size, using transmitted light, and measured with an optical micrometer. The distances from the focus to the edge of the section (spine radius) and from the focus to each growth band were recorded. Measurements were made as shown in Figure 1. The relationship between spine radius and LJFL was determined using standard linear regression procedures. This relationship and the distance from the

[1]University of Miami, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, FL 33149-1099.

[2]University of Maryland, Chesapeake Biological Laboratory, Box 38, Solomons, MD 20688-0038.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

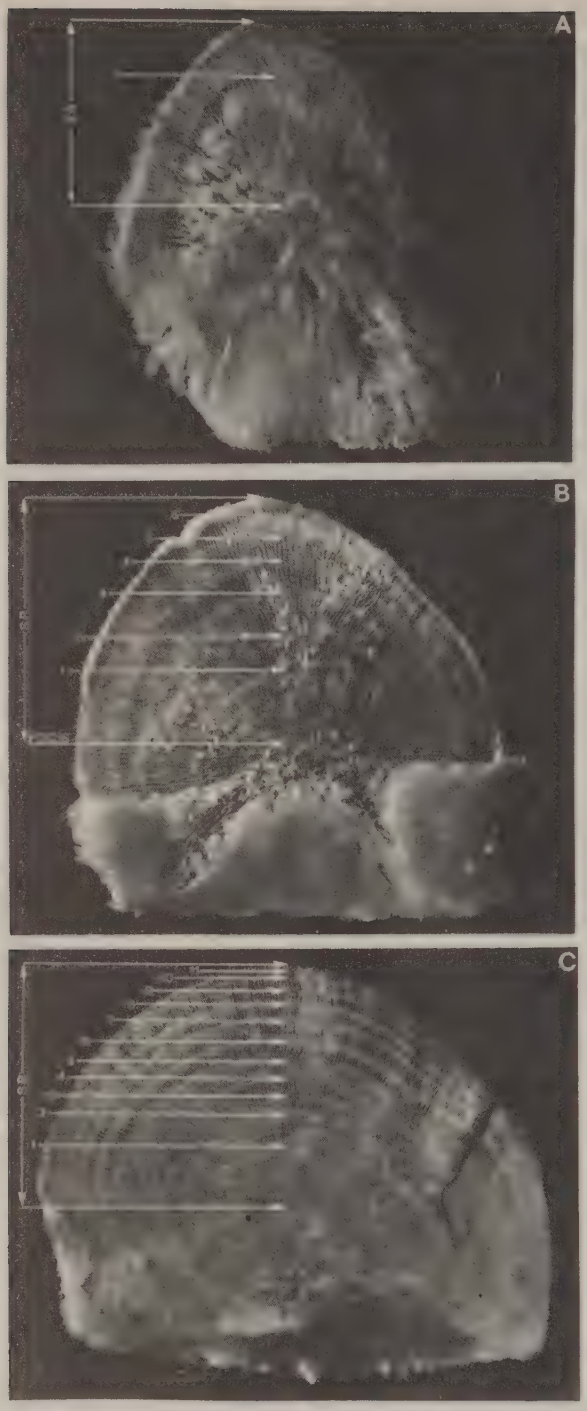

Figure 1.—Typical swordfish second anal fin spine sections. Spine radius (SR) was measured from the focus as shown. Annuli are also shown for: (A) estimated age 1+; (B) estimated age 6+; and (C) estimated age 11+.

focus to successive growth bands, which we assumed to be annual events, were used to back-calculate lengths at presumed age from the relationship:

$$L_n - C = \frac{S_n}{S}(L - C) \quad (1)$$

where L = LJFL at time of capture,
L_n = LJFL when band n was formed,
C = intercept on length axis from regression of length on spine radius,
S_n = distance from spine focus to band n,
S = spine radius.

Mean back-calculated lengths at estimated ages were determined separately for males and females and fit to the von Bertalanffy growth model, using the method of Beverton and Holt (Ricker 1975).

Spine section readability by size class and sex was tested, using a chi-square contingency test.

Time of annulus formation was estimated from mean monthly marginal increments (see Glossary), adjusted for sex and age as follows: The mean distance between successive growth bands was determined by sex for each inter-band distance (i.e., mean distances between bands 1 and 2 were determined for male and female fish with two or more bands, mean distances between bands 2 and 3 for male and female fish with three or more bands, etc.). The marginal increment was then expressed as a percentage of the mean distance between the appropriate bands for fish of the same sex (e.g., for fish of estimated age 2+ the marginal increment was expressed as a percentage of the mean distance between bands 2 and 3, based on all fish of the same sex older than estimated age 3). The adjusted percentage marginal increments were arcsine transformed and the means calculated by month and season. Differences in seasonal means were tested using analysis of variance.

All statistical tests were considered significant if $P \leq 0.05$.

RESULTS

Structure of the Anal Fin Spines

The first anal fin of swordfish is composed of 12-16 hard, spinelike rays (Palko et al. 1981; see Glossary). The first spine is short and stout and is occasionally missing entirely. The second spine is longer and unbranched, while the remaining rays are more compressed, especially near their bases, and are branched distally, often several times. Each fin spine is composed of two closely apposed elements. The central matrix (core) of these elements is vascularized and often contains globules of oil, which occasionally obscure the focus or the first several growth marks. This inner matrix appears to become increasingly calcified as the fish grows older.

Preparing the Element for Reading

After removal of all skin and tissue, the spine was clamped in a vise and sectioned. Sections were found to lose clarity if allowed to dry, presumably because oil contained in the bone would oxidize, turning them opaque and obscuring the growth marks if not placed in preservative. Five percent Formalin was adequate to prevent deterioration and had little effect on the readability of the sections even after several months in storage. Beyond this time, sections stored in Formalin began turning opaque, eventually becoming unreadable, presumably due to the dissolution of calcium by Formalin. Although several

preparation procedures and stains were tried, such as treatment with glycerine, glycerine and water, polarized light, decalcifying solution, and eosin-hematoxylin stain, none improved the readability of the sections.

Characteristics of the Annulus

We define annuli as bands laid down once a year at approximately the same time each year. Bands were presumed to be annuli if they were continuous around the circumference of the entire spine section (see series of spine sections, Fig. 1). When viewed with transmitted light, bands appear as opaque rings (see Glossary). However, when photographed with reflected light (Fig. 1), they appear as light areas. The characteristics of bands varied considerably among individual fish, ranging from a very well-defined, narrow opaque band to a fairly broad diffuse opaque band. Often, the first annulus was difficult to locate and in larger fish it sometimes was completely obscured. Even when visible, the first mark often is diffuse; determining its exact location is often difficult. In older fish, the marks nearest the spine margin are generally the clearest and best defined.

Double or multiple bands were seen often, particularly in older fish. Bands were considered to be double if the distance between them was substantially less than the distance to the preceding and following bands. Often, one of the two double bands did not extend around the entire circumference of the spine. When double or multiple bands were encountered, the clearest band was considered the annulus; auxiliary bands were presumed to be false annuli and were ignored. In some cases, interpretation was impossible and these spines were rejected.

Age-Frequency Distribution

A total of 439 second anal fin spines was collected, sectioned, and measured between 1978 and 1980. Annuli were counted and measured and the percentage frequency of fish in each age class determined (Fig. 2). The oldest fish in our sample was estimated to be 11+ yr, although 61% of the fish were less than estimated age 4.

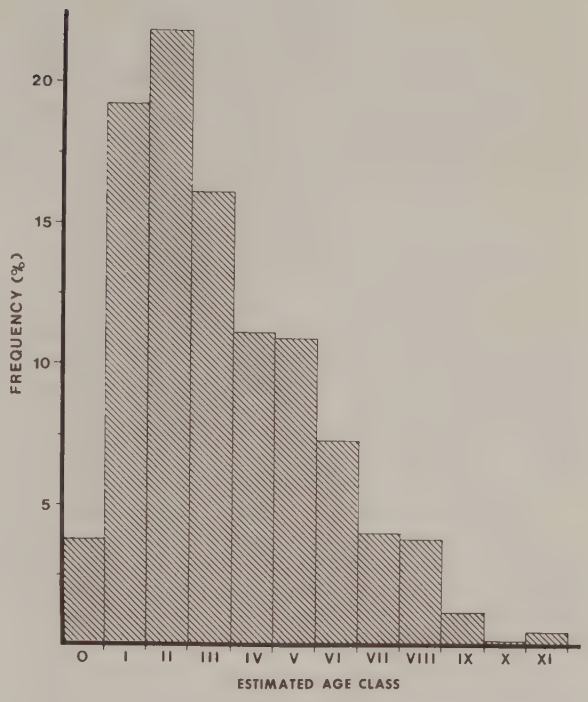

Figure 2.—Age-frequency distribution of 439 swordfish sampled from the Florida Straits, 1978 to 1980.

Fish Size-Fin Spine Radius Relationship

The relationship between LJFL and fin spine radius (Fig. 3) for our sample of 439 swordfish is:

$$LJFL = 58.50 + 23.90S \qquad (r = 0.94)$$

where LJFL = lower jaw fork length (cm),
S = anal fin spine radius (mm),
r = correlation coefficient.

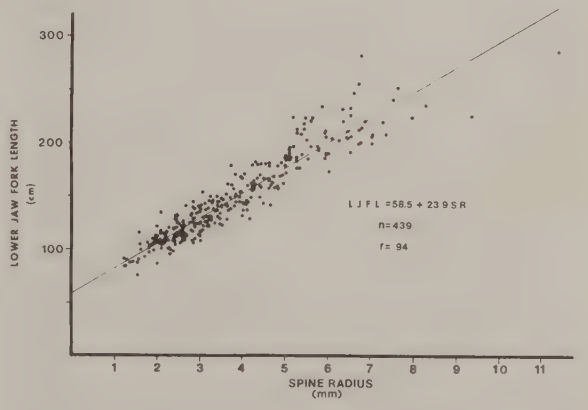

Figure 3.—Relationship between lower jaw fork length and second anal fin spine radius for 439 swordfish sampled from the Florida Straits, 1978 to 1980.

Careful sectioning of each spine at the same position relative to the condyle base helped to obtain the significant linear relationship ($r = 0.94$). There was no significant difference in the fork length-fin spine radius relationship between males and females (t-test; slopes $0.20 < P < 0.50$; elevations $P > 0.50$).

Proportion of Readable Spines

Although virtually all fin spines require some interpretation to be read, there is a wide range among spines in the clarity of their bands. Some spine sections were eliminated immediately as unreadable, occasionally because the sections were too opaque to allow enough light transmission, or more often because the marks were too broad and diffuse to accurately determine their number and location. Occasionally, spines from large fish had a number of distinct outer annuli but bands near the focus were not visible. After reading many sections from fish of all sizes, it often was possible to determine the probable number of missing inner marks by measuring outward from the focus to the first visible mark. If no more than one mark

was believed to be obscured (based on measurements of spines from younger fish) and all other marks were distinct, these spines were considered readable. Sixteen spines (3.6%) fell into this category.

The number and percent of readable and unreadable spines are presented by size class and sex in Table 1. The length classes in Table 1 correspond to the following dressed weights:

LJFL	*Dressed weight*
170 cm	45.4 kg (100 lb)
210 cm	90.7 kg (200 lb)

A chi-square contingency test did not detect significant differences in readability of spines by size class for females ($0.05 < P < 0.10$), but it did detect a significant difference for males ($0.025 < P < 0.05$). The high proportion of unreadable spines (50%) in the small sample (6 fish) in the largest size class (> 210 cm FL) contributed over 82% to the calculated chi-square value for males. Eighty-seven percent of all fish had readable fin spines. It should be noted, however, that readability was subject to interpretation in some cases and probably was influenced by the number of samples available. If a much larger sample had been available, the percentage of spines considered readable might have been smaller.

Table 1.—Readability of swordfish anal spine sections by size and sex.

Lower jaw fork length	Total number	Readable		Unreadable	
		Number	Percent	Number	Percent
Males					
< 170 cm	257	225	87.5	32	12.5
170-210 cm	55	47	85.5	8	14.5
≥ 210 cm	6	3	50.0	3	50.0
All males	318	275	86.5	43	13.5
Females					
< 170 cm	111	103	92.8	8	7.2
170-210 cm	38	30	78.9	8	21.1
≥ 210 cm	36	31	86.1	5	13.9
All females	185	164	88.6	21	11.4
All fish	503	439	87.3	64	12.7

Of the spines considered readable, most required little interpretation after initial criteria and position of the first annulus were established. Spines that appeared usable but not easily read were noted and were re-read at a later date. If the second readings agreed with the first, and the bands met our criteria for annuli, these sections were used for age estimates. If, after the second reading, there was still a question about the location or position of annuli, these spines were considered unreadable and were not used in the age and growth analysis.

Time of Annulus Formation

The distance between the last annulus and the edge of the spine is an indicator of the time of annulus formation. The smaller this distance, the closer the capture date is to the date of annulus formation. If the bands seen on fin spines are valid indicators of age, they should be laid down at approximately the same time each year. Shown in Figure 4 is the mean distance between the last band and the edge of the spine adjusted for age and sex (the adjusted marginal increment) for males, females, and combined sexes by month. An analysis of variance failed to detect significant differences in mean percentage marginal increments among months ($0.10 < P < 0.25$). The adjusted mean marginal increments also were plotted by quarter, beginning in January (Fig. 4). Increments increased steadily from the first quarter of the year, suggesting that annulus formation occurred in winter, but the ANOVA still failed to detect significant differences in means among quarters ($0.10 < P < 0.25$).

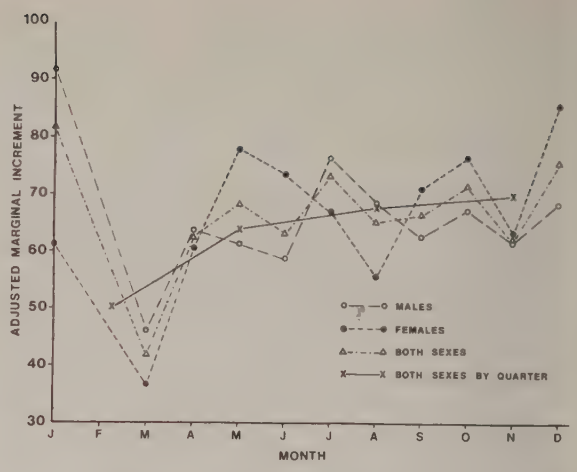

Figure 4.—Mean marginal increment of anal fin spine sections for male, female, and combined sexes of swordfish by month and combined sexes by quarter beginning in January. Marginal increments were adjusted for size and sex and arcsine transformed.

Age and Growth

There is a marked difference in growth rate between male and female swordfish. Females grow faster after estimated age 2 and reach a larger size than males. Mean back-calculated lengths at estimated age were determined separately for male and female swordfish. These data were used to fit the von Bertalanffy growth model (Fig. 5). Equations, parameter estimates,

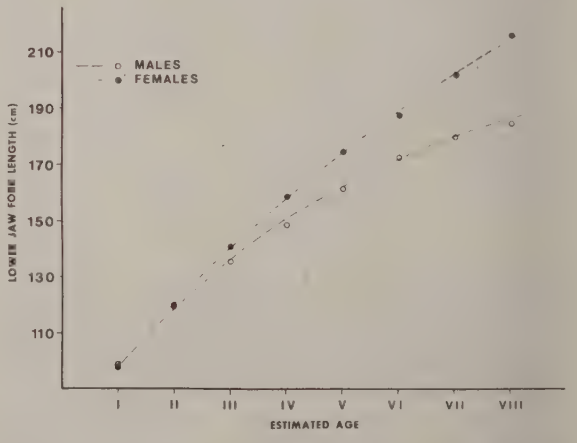

Figure 5.—The relationship between lower jaw fork length (cm) and estimated age (yr) for swordfish landed in south Florida. The data points are back-calculated lengths at presumed annuli for male and female swordfish. Curves are based on the von Bertalanffy model fitted to these data.

Table 2.—Mean back-calculated fork lengths at age and those predicted by the von Bertalanffy model for swordfish landed in south Florida. Equations and parameter estimates also are given.

Estimated age	Males Lower jaw fork length (cm)		Females Lower jaw fork length (cm)	
	Back-calculated	Predicted from von Bertalanffy growth equation	Back-calculated	Predicted from von Bertalanffy growth equation
1	98.86	97.24	97.17	97.99
2	119.27	118.50	119.83	119.85
3	135.41	136.00	140.90	139.73
4	148.54	150.40	158.63	157.82
5	161.55	162.25	174.46	174.28
6	172.79	172.01	187.60	189.25
7	180.38	180.03	202.19	202.87
8	185.12	186.64	216.22	215.25
	$L_t = 217.36(1-\exp[-0.1948(t+2.0444)])$		$L_t = 340.04(1-\exp[-0.09465(t+2.5912)])$	
	$L_\infty = 217.36$		$L_\infty = 340.04$	
	$k = 0.1948$		$k = 0.09465$	
	$t_0 = -2.0444$		$t_0 = -2.5912$	

back-calculated lengths at age, and lengths predicted by the von Bertalanffy model are presented in Table 2.

DISCUSSION

Fin rays and spines, although used less commonly than other hardparts, can be excellent indicators of age in fishes. Beamish (1981) recently reviewed the use of fin spines or rays as age indicators, discussing advantages of the method over more conventional scale or otolith techniques. Annual marks often are more distinct on fin rays and spines than on scales, particularly in older fish (Mills and Beamish 1980; Beamish 1981; Shirvell 1981). We found that annuli on anal fin spines of swordfish were detected easily, even in fish > 10 yr. Illustrations by Jolley (1977) show that annuli are easily detected in sailfish spines as well, and Beamish (1981) advocated use of the method for ageing albacore. The relative ease with which spines can be obtained and the relatively simple preparation procedure are advantages of this method that recommend it for ageing studies on many large pelagic fishes.

One possible disadvantage, which we noted for swordfish and which has been reported for other fishes (Jolley 1977; Beamish 1981; Shirvell 1981), is the loss of the first one or two annuli on spines from older fish. The loss of these annuli reported for other species apparently results from expansion of the vascularized core of the spine during growth of older fish. In swordfish, it appears to result from increased calcification of the spine near the focus. This problem did not necessarily prohibit age determination because experienced fin spine readers recognize the situation and usually can determine when an annulus has been obscured, based upon their knowledge of location and formation of growth bands in spines from younger fish. We found marks on swordfish anal fin spines other than those that we interpreted to be annuli. These marks usually did not completely encircle the spine and often appeared too close to the preceding annulus to be considered a year mark, thus appearing as a double mark.

The high percentage of readable spines (87%) partly resulted from the relatively small number of spines available to us for analysis. If more samples had been available, there would have been less incentive to accept readings from spines requiring a great deal of interpretation in analysis. However, assuming that errors were random (some under- and some over-ageing), we believe that our results have not been biased seriously. The significant difference in readability of spines for males resulted from the high proportion of unreadable spines in the largest size category (> 210 cm LJFL). Although the sample of males in this size class was small, the results of the chi-square contingency test imply that large males are the most difficult to age from fin spines. This probably results from the slower growth rate of males, resulting in closely spaced annuli and therefore increased difficulty in interpreting spine sections from older fish.

Evidence indicates that the bands on spines, which we believe to be annuli, are probable indicators of age. Results from our ageing study, which were used to back-calculate lengths at age and fit the von Bertalanffy growth model, yielded parameter estimates that seem consistent with what is known about the life history of the species. The difference in sizes of males and females, with females being larger, is well documented (Cavaliere 1963; Guitart-Manday 1964; Kume and Joseph 1969; Berkeley and Houde 1981). Beckett (1974) suggested that few male swordfish exceed 200 cm LJFL, and we agree with him. There were eight males longer than 200 cm LJFL (3% of all males) in our samples, the largest being 214 cm LJFL. Radtke and Hurley (1983), in a sample of 303 swordfish, found one male over 200 cm LJFL (208 cm). Considering this, our estimate of L_∞ for males of 217.4 cm LJFL seems reasonable.

The largest swordfish caught on rod and reel weighed 1,182 lb (536.2 kg). This fish measured 350 cm LJFL, and must have been female. Because this fish is near the maximum documented size, our estimate of L_∞ = 340 cm for females also is reasonable. Growth rates predicted by our growth model agreed well with rates determined from tag recaptures.[4] In addition, extensive series of size-frequency data from the Brazilian longline fishery show length modes in the catches that agree closely with our size-at-age estimates (footnote 4). The age-frequency distribution of fish in Florida catches, derived from our ageing

[4]International Commission for Conservation of Atlantic Tunas (ICCAT). 1981. Draft report of the ICCAT inter-sessional workshop on billfish, held in Miami, Florida, 15-19 June 1981, 50 p.

method, seems reasonable for a large, moderately long-lived fish (i.e., > 10 yr) like the swordfish.

Although the analysis of variance did not detect a significant difference in season of annulus formation, mean marginal increments did show a trend, being smallest in winter and becoming progressively larger during the year. While it appears that late winter is the season of annulus formation for most swordfish in the Florida fishery, there is considerable variability among individuals. In addition, small sample sizes available in winter compared with other seasons may have contributed to the inability to differentiate statistically the seasonal differences. Jolley (1977) found that most sailfish from Florida waters formed annuli during fall and winter, but he also had interpretation problems because of individual variability in time of annulus formation. The factors responsible for annulus formation are not known at this time but may be related to complex migratory patterns of the fish. The variability could be a reflection of individual or stock-specific behavior. Swordfish are widely distributed in the Atlantic but spawning is confined to the tropics and subtropics (Palko et al. 1981), with the Gulf of Mexico and Straits of Florida being major spawning areas (Markle 1974; Grall et al. 1981). Therefore, swordfish caught in the Straits of Florida may represent populations or stocks from widely divergent geographic areas and thus may be subject to very different seasonal temperature and feeding regimes, resulting in variability in time of annulus formation.

Although fin spines apparently offer a good method to age swordfish and other billfishes, there is a need to validate the method independently. Two recent studies on age and growth of swordfish, using otoliths as the ageing structure (Radtke and Hurley 1983; Wilson and Dean 1983), yielded results that differed from ours (Table 3). Both of those studies suggest slower growth rates than we estimated (after age 3) from bands on anal fin spines, but the results were not consistent with each other. Wilson and Dean (1983) reported good agreement on age estimates from fin spines read by S. Berkeley and otoliths taken from the same fish, but the length-at-age estimates in the two studies, although similar, do not agree. Clearly, there remains a need for additional ageing work on these fish.

Successful ageing of swordfish is a critical step in studies on population dynamics, which are necessary for management of Atlantic Ocean populations. We have used our age estimates in an analysis of stock dynamics (Berkeley and Houde 1981). Growth models, mortality estimates, and yield models were derived, which were dependent upon accurate ageing of swordfish. Although there remains a degree of uncertainty in ageing based on anal fin spine analysis, we are reasonably confident that ages were assigned with enough accuracy to allow a good preliminary assessment of swordfish stock dynamics in the Florida Straits. Continued efforts to obtain accurate age and growth estimates of swordfish, including hardpart analysis of tag return data from swordfish of known size at time of tagging and at recapture, will be useful to validate our estimates and improve the assessment analysis for future management needs.

Table 3.—Length-at-age estimates of broadbill swordfish from Straits of Florida (Berkeley and Houde 1981), North and South Carolina (Wilson and Dean 1983), and North Atlantic Ocean (Radtke and Hurley 1983).

Estimated age	Lower jaw fork length (cm)					
	Berkeley and Houde (1981)		Wilson and Dean (1983)		Radtke and Hurley (1983)	
	Males	Females	Males	Females	Males	Females
1	97.2	98.0	116.9	122.9	84	73
2	118.5	119.9	123.3	130.6	98	95
3	136.0	139.7	130.2	138.8	110	114
4	150.4	157.8	137.4	147.5	122	131
5	162.3	174.3	145.0	156.8	133	147
6	172.0	189.3	153.0	166.6	143	160
7	180.0	202.9	161.5	177.1	153	172
8	186.6	215.3	170.4	188.2	161	183

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Age Estimation and Growth of Broadbill Swordfish, *Xiphias gladius*, from the Northwest Atlantic Based on External Features of Otoliths

RICHARD L. RADTKE[1] and PETER C. F. HURLEY[2]

ABSTRACT

Age and growth were estimated based on external features of otoliths from 303 broadbill swordfish, *Xiphias gladius*, captured by longline from Cape Hatteras to the Grand Banks of Newfoundland during the summer and fall of 1980. Scanning electron microscope examination of whole and sectioned sagittae otoliths revealed well-defined external ridges and finely spaced internal increments. These finely spaced internal increments were similar to those found in other fish species and their total number in two swordfish support annual formation of the external ridges. Lower jaw fork length (LJFL)-at-age estimates were analyzed using the von Bertalanffy growth equation and produced estimates of k, t_0, and L_∞ of 0.07, −3.94 yr, and 277 cm LJFL for males and 0.12, −1.68 yr, and 267 cm LJFL for females, respectively. Our estimates of age from external features of swordfish otoliths suggested slower growth rates than other research on this species based on anal fin spines, internal features of otoliths, or modal analysis of size frequencies.

INTRODUCTION

The swordfish, *Xiphias gladius*, has worldwide distribution (Rich 1947; Wise and Davis 1973; Palko et al. 1981) and is limited to waters with temperatures above 13°C in the Atlantic (Tibbo et al. 1961; Beckett 1974). This species supports important commercial and recreational fisheries throughout its range. Research in the Atlantic has shown that swordfish may be susceptible to overfishing; however, a lack of biological data, particularly of swordfish growth rates, has hampered attempts to apply standard analyses used in population dynamics (Beardsley 1978).

Reports of age estimates and growth rates of swordfish are many and appear to vary according to the method of ageing used, the geographical location, and size and range of samples obtained. Beckett (1974), using weight frequencies from the Canadian swordfish fishery, together with examination of vertebral rings and tagging data, suggested a rapid growth rate for female swordfish with weights of 4, 15, 40, 70, and 110 kg for ages 1-5 yr old. Caddy (1976) converted these data to lower jaw-fork length (LJFL) ranges and reported 50-90, 100-110, 120-150, 160-180, 190-220, and 230-260 cm for swordfish 1-6+ yr old. Ovchinnikov et al. (1980), using modal analysis of length frequencies of swordfish taken in the Soviet tropical Atlantic fishery, reported mean eye orbit-fork lengths (EOFL) of 65, 90, 110, 140, 150, 170, 200, and 210 cm (sexes not differentiated) for swordfish 1-8 yr old.

Guitart-Manday (1964) suggested that a swordfish 160 cm long taken off Cuba was 2 yr old. Using seasonal progression of size modes, Yabe et al. (1959) found that swordfish in the western Pacific 50-60 cm EOFL were 1 yr old and grew about 25 cm/yr, while Kume and Joseph (1969) reported that swordfish in the eastern Pacific 62-165 cm EOFL grew about 38 cm/yr. Beckett (1974) suggested that few males exceed 200 cm LJFL.

Scales are absent in adult swordfish and Beckett (1974) reported that growth bands observed on vertebrae and operculae did not produce interpretable results. Artüz (1963) reported what he believed to be annual marks in the dorsal fin spines of swordfish. Berkeley and Houde (1981, 1983) estimated the age of swordfish taken in the Straits of Florida, using growth bands observed on anal fin spine sections. They found different growth rates for males and females, as suggested in previous studies (Cavaliere 1963; Guitart-Manday 1964; Kume and Joseph 1969; Beckett 1974; Skillman and Yong 1974), and reported estimates of von Bertalanffy growth parameters k, t_0, and L_∞ of 0.19, −2.04 yr, and 217 cm LJFL for males and 0.09, −2.59 yr, and 340 cm LJFL for females, respectively.

Otoliths have not been used in ageing studies of swordfish in the past due to their minute size (Beckett 1974). Recently, through the application of the scanning electron microscope (SEM), the use of otoliths in estimating the age of swordfish (Wilson and Dean 1983) and other billfish species (Radtke 1983) by counting internal increments has shown promise. In this study, we employed SEM techniques to describe the internal and external morphology of sagittae otoliths from swordfish collected from the northwest Atlantic, and counted external ridges on sagittae to estimate age and growth rates.

MATERIALS AND METHODS

A total of 303 swordfish were collected during four cruises conducted by the St. Andrews Biological Station in the summer and autumn of 1980 (Fig. 1). The sex of each specimen was determined by gross examination of the fresh gonads and the LJFL was measured with calipers (±0.1 cm). The swordfish were decapitated by a vertical cut through the posterior

[1]University of Hawaii at Manoa, Hawaii Institute of Marine Biology, P.O. Box 1346, Coconut Island, Kaneohe, HI 96744.

[2]Department of Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick Canada; present address: Marine Fish Division, Fisheries Research Branch, Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2.

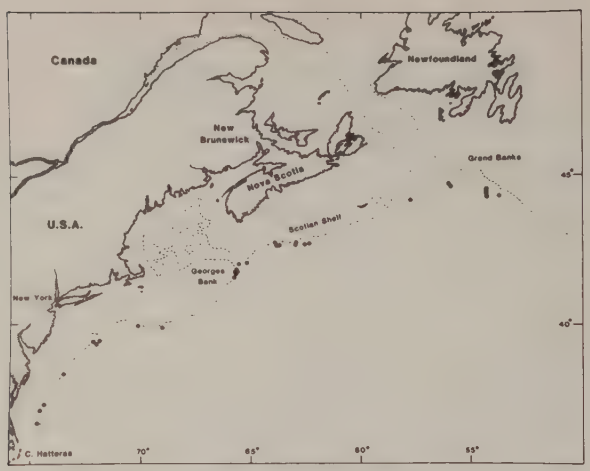

Figure 1.—Fishing locations for 1980 swordfish survey.

margin of the preoperculum and the heads frozen for later dissection. Otolith extraction and SEM preparation techniques following Radtke (1983) were used in this study.

Detailed observations of external otolith morphology were made with a SEM (25-5,000×). The morphological nomenclature of Hecht (1978) and Morrow (1979) was used to describe the sagitta. An age estimate was assigned to each specimen based on counts of the number of laminations or ridges on the surface of the rostral lobe of the sagitta otolith, as observed by a single reader (Radtke). Three counts were made for each left sagitta otolith in the following fashion: 1) Sagittae were given identification numbers and randomized after being attached to SEM stubs, 2) three SEM counts were performed for each sagitta with the sagittae randomized before each independent count, 3) if all three ridge counts differed, the otolith was rejected; if two or more of the ridge counts were identical, that count was accepted. The von Bertalanffy growth equation was fitted to these length-at-age estimates for males and females separately, using the procedure of Allen (1967) to estimate growth rate.

Fifteen sagittae otoliths were cut in a transverse plane through the core region with a low-speed rock saw and prepared for SEM examination as described in Radtke (1983) to view internal otolith morphology. Finely spaced increments (assumed to be daily), concentric around the core region and radiating to the otolith edge, were observed on the otolith sections. Otoliths were etched using EDTA (disodium ethylenediaminetetraacete). Differential etching by EDTA occurred, with short etching times intensifying the outermost increments and progressively longer etching times enhancing the inner increments. Scratches were made in the surrounding epoxy of each section as reference marks. Through a series of increasingly longer etching intervals, with repolishing of the section between each interval, it was possible to locate the core region. This procedure allowed us to obtain increment counts for the entire otolith section, by using the reference marks to accurately orient the clearly etched area during each count. Each area was counted 10 concurrent times by a single reader (Radtke), as it was made visible by difterential etching. In 13 samples, the core region could not be established or increments could not be discerned in some areas of the section; however, in two cases it was possible to establish the core region and enumerate all increments over the entire section. The results from the counts of finely spaced increments were then compared with the age estimate produced by a count of the external ridges observed on the whole otoliths before sectioning.

RESULTS

External Morphology

The otoliths of swordfish, like other billfish (Radtke 1983), were notably small. The sagittae otoliths examined ranged from 1.5 to 2.8 mm in rostral length. The lapillus and asteriscus otoliths were much smaller than the sagitta and the lapillus was thicker than the asteriscus; the latter was very fragile and often fractured upon dissection. Thus, we judged the sagitta most useful for ageing.

In order to correctly use external features of the sagitta for age estimation, it was necessary to recognize the otolith's topographical relief. The sagitta (Fig. 2) has a well-demarcated rostrum, a distinct sulcus, and was devoid of a collum and anterior and posterior cristae. In most sagittae, the rostrum was exaggerated with the antirostrum being smaller than the rostrum. The antirostrum of sagittae increased in size as fish size increased. The anterior excisural notch was very distinct in all specimens, while a distinct posterior notch was present only in some specimens. In small specimens, the posterior excisural notch manifested itself as an aperature in some specimens and was entirely absent in others. The incidence of aperatures was greater in smaller specimens. In those specimens lacking a posterior excisural notch, the rostrum and antirostrum were joined to form a cup-shaped arrangement.

Concentric laminations or ridges, radiating from the core region, were observed on all three otoliths. Ridges were most prominent on the surface of the rostrum of the sagitta (Fig. 2), and the counting path was made on this lobe for age estimation. However, in some cases it was necessary to use several profiles or the antirostrum because of the occasional twisted nature of the otolith and to provide a better three-dimensional view. The large degree of curvature and variability in shape of sagittae precluded measuring the size of ridge radii. Therefore, back calculation of lengths could not be attempted.

Internal Morphology

Sections of sagittae otoliths exhibited internal structures, suggesting incremental growth (Fig. 3). Finely spaced increments, concentric around the core regions, were present and it was possible in two samples to observe these increments from the core region to the otolith edge. In one case, the intact sagitta otolith of a female swordfish with an LJFL of 156 cm exhibited six ridges on the surface of the rostrum and was consequently estimated to be 6 yr old (Fig. 2). A transverse section of the same otolith displayed 2,302 ± 18 finely spaced internal increments. In a second case, the intact otolith of a female with an LJFL of 195 cm showed nine ridges, while a section revealed 3,352 ± 9 internal increments. No indication of the external ridges was observed in the transverse sections.

Age and Growth Estimates

Of 303 swordfish samples, 24 (7.9%) were found unsuitable for ageing analyses and were rejected. In addition, the age esti-

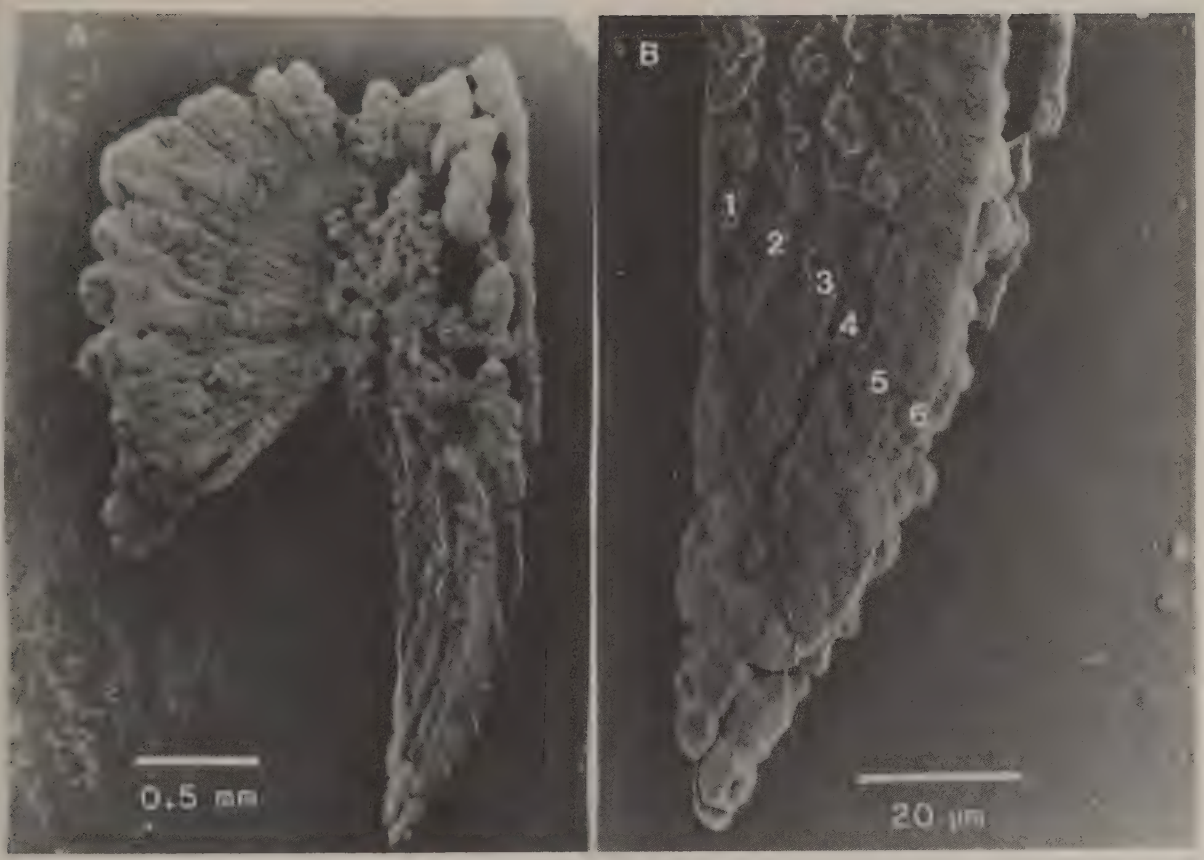

Figure 2.—(A) Sagitta from a 156 cm lower jaw fork length swordfish estimated to be 6 yr old; (B) magnified view of the rostrum showing ridges used in estimating age.

mates of 11 other specimens were dropped from the analysis since either fish length or sex had not been determined. The observed size range was 88-208 cm LJFL for 73 males and 80-280 cm LJFL for 195 females, while the corresponding range in age estimates for these specimens was 2 and 14 yr for the males and 2 and 32 yr for the females, respectively. Parameter estimates for the von Bertalanffy growth equation were $k = 0.073$, $t_0 = -3.94$ yr, $L_\infty = 277.2$ cm LJFL for males and $k = 0.120$, $t_0 = -1.678$ yr, $L_\infty = 266.7$ cm LJFL for females (Table 1). Fits of the observed data to the equation indicated a highly variable growth rate for both sexes but the von Bertalanffy growth model had a better fit to the observed data for females than for males (Fig. 4).

DISCUSSION

External Morphology

The otoliths of swordfish were morphologically different from those described for other billfish species (Radtke 1983). Otolith morphology has been postulated to be species-specific (Hecht 1978; Morrow 1979). The otoliths of swordfish appear species-specific, but large intraspecific variations in otolith morphology were displayed in the specimens from the northwest Atlantic examined in this study. Otolith structures and morphology have been employed to discriminate herring stocks (Messieh 1972) and steelhead trout populations (Rybock et al. 1975). Thus, otolith morphology of swordfish may be a useful tool in stock discrimination.

SEM studies of otoliths of other species have shown external ridges similar to those observed in this study (Radtke and Dean 1981; Radtke et al. 1982; Wilson et al. 1982). If the ridges observed in this study are related to an annual periodicity, their use in swordfish ageing would provide a fairly reliable technique (7.9% rejection rate). Although swordfish otoliths are very small and require careful and precise dissection and handling techniques, the use of external morphological features would preclude sectioning preparation.

Internal Morphology

Recent studies of otoliths from many species have confirmed that daily increments can be employed to determine growth patterns (Brothers et al. 1976; Struhsaker and Uchiyama 1976). If the finely spaced increments observed in the sections of otoliths are assumed to be daily, the counts of these increments made in two cases indicate that these specimens were approximately 6.3 and 9.2 yr old. Based on counts of the external ridges, these specimens had been assigned estimated ages of 6 and 9 yr old, respectively. Therefore, the close agreement ob-

Figure 3.—(A) Finely spaced increments in a medial section of swordfish otolith; (B) magnified view of finely spaced increments.

Table 1.—Parameter estimates, with 95% confidence intervals, derived from the von Bertalanffy growth equation for 73 male and 195 female swordfish from the northwest Atlantic, 1980.

	Males			Females		
Parameter		Lower	Upper		Lower	Upper
k	0.073	0.021	0.125	0.120	0.103	0.136
t_0	−3.942	−6.245	−1.640	−1.678	−2.205	−1.150
L_∞	277.183	187.685	366.681	266.663	254.594	278.732

served in these two specimens between the estimated age in days as determined by finely spaced internal increments and the estimated age in years as determined by external ridges support an interpretation of the later as a source of annual age and growth information. However, since no internal structure corresponding to the external ridges was observed, there is no direct structural evidence that the internal increments and the external ridges are correlated, other than the agreement in these two cases.

Age and Growth Estimates

The age estimates produced using this technique suggest a maximum lifespan of at least 14 yr for male and 32 yr for female swordfish from the northwest Atlantic. A different growth rate for males and females was indicated in this study, as has been shown in previous studies (Cavaliere 1963; Guitart-Manday 1964; Beckett 1974; Berkeley and Houde 1981). The results indicated that females grow faster than males after estimated age 2, as reported by Berkeley and Houde (1981). In addition, our estimated growth rates are much lower than those produced by modal analysis of size frequencies (Guitart-Manday 1964; Beckett 1974; Caddy 1976; Ovchinnikov et al. 1980), examination of anal fin spines (Berkeley and Houde 1981, 1983), or examination of internal otolith structure (Wilson and Dean 1983). These discrepancies cannot be fully explained at this time but could be related to differences in techniques, stocks, range and size of samples, or a combination of these or other factors.

The fit of the data to the von Bertalanffy growth equation was much better for females than for males and, in fact, suggested a large degree of variability in growth rate for both sexes. This variability may be due to several factors: Unsuitability of the model, inaccuracy of the technique, or variability in growth rates between individuals, between cohorts, or both. The data were not analyzed using other growth models and there is no suggestion that the von Bertalanffy is the most suitable.

Several shortcomings of the model should be discussed. The von Bertalanffy parameters, k and L_∞, are inversely related

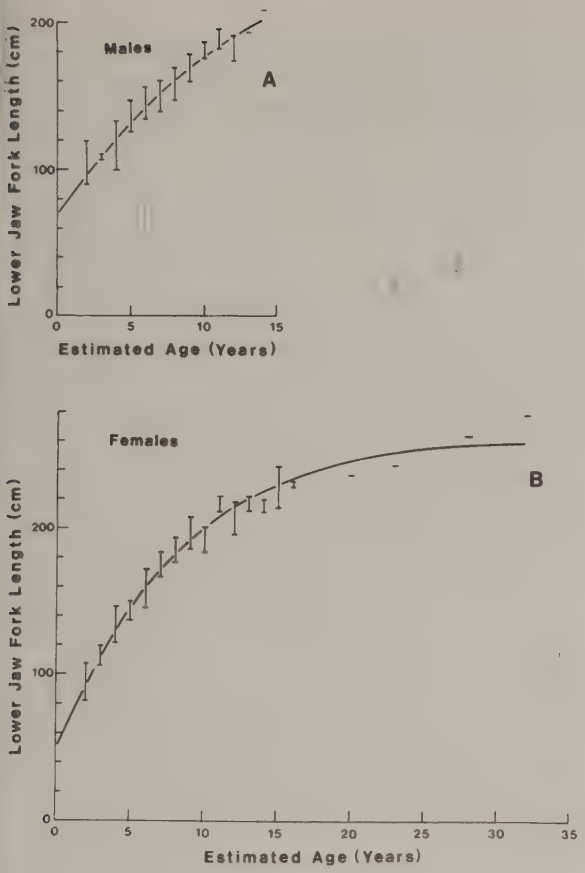

Figure 4.—Von Bertalanffy growth curve from length-at-estimated age data for male (A) and female (B) swordfish. Vertical rules indicate ± standard deviation, dashes indicate single observation.

and are affected by both range and distribution of data (Southward and Chapman 1965). Very small and very large fish were not well represented in this study, and a lack of data at the extremes of the size range may have had an effect on the resulting von Bertalanffy parameter estimates. While Knight (1968) cautions against comparing L_∞ values with observed maxima, the L_∞ value found here for males is much larger than the reported maximum, while that for females is much smaller than the reported maximum.

In addition to sample coverage, errors in assigning ages may also affect the accuracy of parameter estimates. Overestimation of age would tend to lower L_∞ and raise k, while underestimation of age would tend to raise L_∞ and lower k. While the agreement between the ages estimated by internal increments and external ridges in the two cases supports the assumption that the ridges have an annual periodicity, there is no direct evidence that this is so.

There is also the potential for the age estimates to be biased since there was only one reader in this study. However, multiple independent readings with randomization between readings were employed to minimize this possibility. The use of only one reader also limited our ability to measure precision of the age estimates. Consideration of marginal increments and back calculation of lengths from external ridge radii measurements might have been effective in reducing variability in the results, but unfortunately the curvature of the surface of the sagittae precluded any measurements.

While our technique shows promise as a method of ageing swordfish, these results are preliminary and further work is necessary. Two other papers on swordfish (Berkeley and Houde 1983; Wilson and Dean 1983) are also in agreement in this regard. Extending sampling to the extremes of the data range may alter the von Bertalanffy parameter estimates; however, a high degree of variability in growth rates between individuals and between cohorts may be inherent in a fast-growing, long-lived, oceanic pelagic species that migrates great distances. Validation of ageing techniques (Brothers 1983) through analysis of hardparts from a tagged fish of known size, age, and sex, or from tetracycline marking experiments, is essential, particularly if variability in growth rate is high.

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The Potential Use of Sagittae for Estimating Age of Atlantic Swordfish, *Xiphias gladius*[1]

CHARLES A. WILSON and JOHN M. DEAN[2]

ABSTRACT

Otoliths (sagiitta, lapillus, and asteriscus) from 121 Atlantic swordfish, *Xiphias gladius*, were collected from commercial vessels along the Atlantic coast between Cape Hatteras and Florida during 1981, for use as a possible source of age information. Internal and external characteristics of sagittae were examined with scanning electron and light microscopy to evaluate those features that showed potential for age estimation. Incremental growth (assumed to be daily and annual) was observed along the lateral edge in a transverse plane of sectioned swordfish sagittae. Counts of such increments in juveniles and between slow-growth zones in young swordfish (1-2 yr) support the assumption of annual zone deposition. In addition, good agreement (91%) between counts of presumed annual growth increments on sagittae and counts of annular bands on sectioned anal spines collected from the same fish (45) support the occurrence of annual events on sagittae and verify their use for age estimation of swordfish. Age estimates made from counts of assumed annual and daily growth increments on sagittae sections ranged from 50 d to 15 yr.

INTRODUCTION

Otoliths are one of several skeletal hardparts used by biologists for determining the age of fishes. The sagitta, usually the largest of three otoliths (Popper and Coombs 1980), has been the most common one used for ageing purposes. Since Panella's (1971) work on the microstructure of fish otoliths, microscopic examination of sagittae has shown that the translucent and opaque zones are composed of daily increments (Brothers et al. 1976; Struhsaker and Uchiyama 1976; Barkman 1978). The majority of this work has been on larval and juvenile fish with relatively short life spans, whereas attempted studies on long-lived or large pelagic species have not been as successful.

Age estimation has been difficult in billfish (Istiophoridae and Xiphidae), particularly with otoliths. Earlier studies concluded that billfish otoliths were too small (Beckett 1974), unclear for ageing purposes (Iversen 1955; Ovchinnikov 1970), or without growth zones recognizable as annual events. They provided no description of the otolith morphology and only general comments on their size. Recent detailed descriptions by Radtke and Dean (1981) and Radtke et al. (1982) of otoliths in sailfish, *Istiophorus platypterus*, and Atlantic blue marlin, *Makaira nigricans*, suggested that some external and internal features of sagittae were in fact useful for age estimation.

In this report we describe the morphology of the otoliths (particularly sagittae) of swordfish and we evaluate the assumed daily and annual growth information present in sagittae and estimate the age of 78 fish.

MATERIALS AND METHODS

Otoliths (n = 121) and anal spines (n = 56) of swordfish were collected during commercial swordfishing operations along the coasts of North and South Carolina, Georgia, and Florida in the summer of 1981. Total weight, lower jaw fork length (LJFL), and sex were recorded for each fish.

Semicircular canals (n = 121) were removed and preserved in 100% ethanol (Radtke and Dean 1981; Haake et al. 1982). Otoliths were later removed from the tissues, cleaned in Chlorox (5.25% sodium hypochlorite), rinsed in xylene, 95% ethanol, and air dried. Sagittae from 81 fish were recovered from the 121 fish sampled. Sagittae were sometimes lost during sampling because the saccular portion of the semicircular canal (containing sagittae) broke off and remained lodged in the skull when the canal was removed. Weights of the sagittae (n = 81 pairs) were measured to 0.001 mg (± 5%) using a Perkin Elmer[3] AD2Z ultramicrobalance.

A power function was used to describe length-weight relationships of both sexes. Comparisons of data were performed by either analysis of covariance, analysis of variance, or Student's t-test (Ott 1977). All statistical inferences were made with a significance level of $\alpha = 0.05$. The otolith-weight/fish-weight relationship was examined fitting, by linear least squares, the natural log (ln) transformation of the following power function:

$$W_f = bW_o^m \quad (1)$$

where W_f = fish weight (kg), W_o = otolith weight (mg), and b and m are equation parameters.

The surface morphology of the otoliths was examined with a scanning electron microscope (SEM) using the method of Haake et al. (1982). The sagitta, which is the largest of the three otoliths in the swordfish, appeared to have the most promise for age determination and it was used for examinations of the internal morphology. Sagittae were embedded in epoxy resin (Spurr 1969), sectioned in the transverse plane on a Buehler Isomet saw, and polished to 0.5 mm thickness with 600 grit sandpaper and 0.3 μm alumina polish. Three of the 81 samples were lost during preparation; hence, 78 samples were used for age esti-

[1]Contribution No. 528 from the Belle W. Baruch Institute for Marine Biology.

[2]Belle W. Baruch Institute for Marine Biology and Coastal Research, Department of Biology and the Marine Science Program, University of South Carolina, Columbia, SC 29208.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

mation. Sections were decalcified with 5% EDTA (disodium ethylenediaminetetraacete, pH 7.5), mounted on aluminum stubs, and examined with either a JEOL SMU3 or JSM 35 scanning electron microscope at 25 kV. In addition, sections were examined at 600 to 1,500× with a light microscope.

Age estimations from otoliths were based on counts of "rapid-growth zones" and "slow-growth zones" described by Irie (1960), which resulted in different light refraction patterns as observed in a thin transverse section of sagittae. Two zones were differentiated: Translucent zones (assumed to be formed during the summer) through which transmitted light passed freely and opaque zones (assumed to be formed during the winter) through which transmitted light did not readily pass. Age estimates were assigned to each sample by counting opaque zones, which we assumed were annuli (see Glossary), formed once each year.

We attempted to verify age estimates from sagittae by comparing them with age estimates made from the second anal spines of the same fish. The first, second, and third elements of anal fins were excised from swordfish (n = 56, 45 of which could be analyzed), cleaned of tissue, and placed on ice while aboard fishing vessels. The second spine was determined to be the best for ageing swordfish landed in Florida (Berkeley and Houde 1983), so it was separated from the remaining elements in the 45 useful samples and either sectioned or given to S. A. Berkeley[4] in 1982 (n = 11) for verification of age estimates. Spines examined in our lab (n = 34) were air-dried, sectioned on the Isomet saw (2 mm), and observed on a dissecting microscope with transmitted light (Berkeley and Houde 1983). A series of alternating dark and light rings roughly concentric with the center of the spine sections, as described by Artüz (1963), Jolley (1977), and Berkeley and Houde (1980, 1983), were observed on sections of spines. The dark rings (opaque zones) were counted and assumed to be annuli.

[4]S. A. Berkeley, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149.

RESULTS

Otolith Morphology

Swordfish sagittae (Fig. 1a) ranged from 1 to 6 mm in length and were 0.1-0.5 mm wide. The medial surface was concave or cup-shaped with a deep prominent sulcus (measured as the distance from the core to the edge of the rostrum in transverse section) that increased with fish size. The rostrum was longer than the antirostrum in the anterior direction and its length increased with fish size; the posterior margins were joined. The cup-shaped feature of the sagittae was formed by the rostrum, antirostrum, and sulcus, and consisted of lobes that radiated from the core toward dorsal, ventral, and posterior surfaces. We observed that while the posterior portion of the sagittae in larger fish was completely calcified, in smaller fish it frequently was not. The anterior portion was always open.

Length-Weight Relationships

Swordfish males ranged from 79 to 209 cm LJFL, while females were from 102 to 290 cm LJFL (Table 1). There was a significant difference between the LJFL of females and males when all data were compared (ANOVA, P = 0.05). The length-weight relationship for male swordfish, $W = 1.13 \times 10^{-6.47}$ (r = 0.88), was significantly different (ANCOVA, P = 0.01) from that of the females whose regression was $W = 7.12 \times 10^{-6.08}$ (r = 0.93).

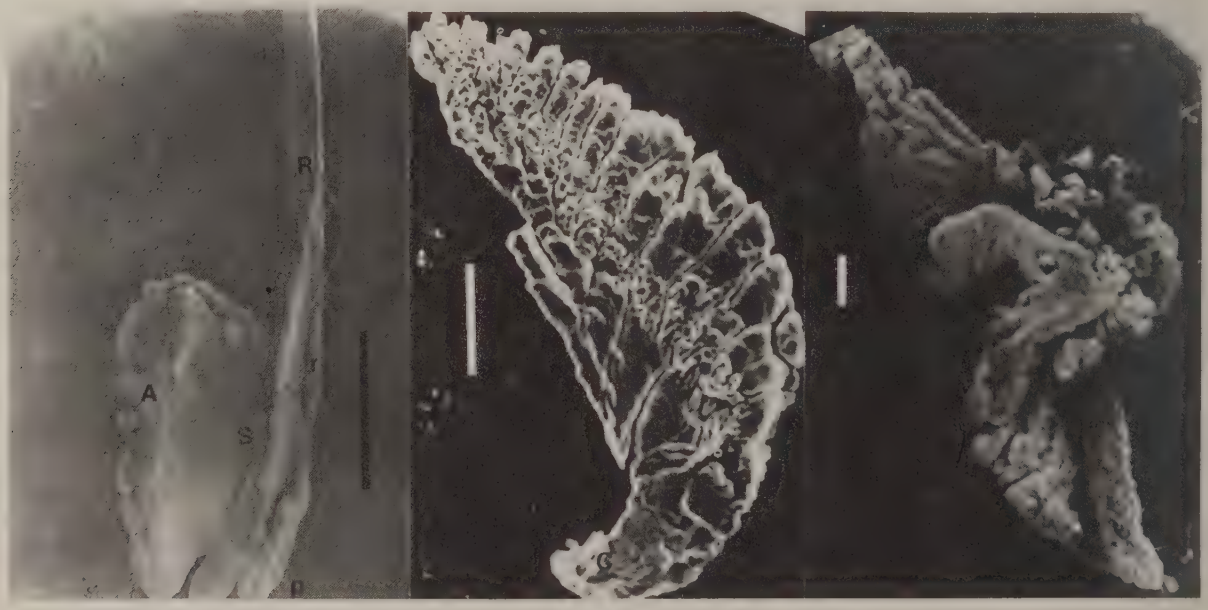

Figure 1.—(A) Scanning electron micrograph of a sagitta from a 72.5 kg (160 lb) Atlantic swordfish, medial view (R = rostrum, A = antirostrum, S = sulcus, r = ridge, a = anterior, p = posterior, bar = 0.5 mm); (B) Scanning electron micrograph of an asteriscus from a 108.9 kg (240 lb) swordfish, medial view (c = core, bar = 250 μm); (C) Scanning electron micrograph of a lapillus from a 108.9 kg swordfish, laterial view (bar = 15 μm).

Table 1.—Mean, sample size (N), standard deviation (SD), range of body weights (kg), body lengths (LJFL), otolith weights (mg), and otolith weight/fish weight ratio for sexed swordfish collected during 1980 and the spring of 1981 along the Atlantic coast from Cape Hatteras to Florida.

	Male			Female		
	N	Mean ± SD	Range Min – Max	N	Mean ± SD	Range Min – Max
Body weight	38	22.870 ± 15.580	1.810 – 67.150	30	33.130 ± 41.110	9.980 – 242.720
Body length (LJFL)	51	128.880 ± 23.550	79.000 – 209.000	46	145.670 ± 36.820	102.000 – 290.000
Otolith weight	43	0.881 ± 0.392	0.360 – 2.190	38	1.030 ± 0.490	0.390 – 2.680
Otolith weight / Fish weight	38	0.037 ± 0.014	0.008 – 0.083	30	0.038 ± 0.014	0.008 – 0.063

Sagittae Weight-Fish Weight Relationships

Weights of swordfish sagittae (n = 81) ranged from 0.36 to 2.19 mg and 0.39 to 2.68 mg for males and females, respectively (Table 1). The best linear relationship was between sagittae weight (sexes pooled) and ln transformed fish weight, but this relationship was not significant (r^2 = 0.27). However, within each sex there was a general increase in otolith weight with an increase in fish weight.

Age Estimation

The external surface of the rostrum of swordfish sagittae lacked the consistent and discrete ridge formations that have been used to estimate age in other billfish (Radtke and Dean 1981; Radtke et al. 1982; Radtke 1983); therefore, we examined internal features for characteristics potentially useful for age estimation. Observation of a sagitta from a 90.05 mm postlarva (Fig. 2) and another small swordfish (27 cm LJFL) revealed increments similar to daily increments described in other species (Tanaka et al. 1981). Increments were easily observed in a transverse section of the sagitta from a 49 kg swordfish (Fig. 3) and were uniform and quite distinct along the lateral edge of the rostrum and occasionally along the width of the antirostrum. Each increment was composed of a discontinuous zone and an incremental zone, which were described by Tanaka et al. (1981) to be formed each 24 h in *Tilapia nilotica*. Increments near the core were 5-10 μm wide and decreased in width towards the medial edge of the rostrum where they ranged from 0.1 to 1 μm in width. Since increments revealed in a transverse plane along the rostral edge section were similar to previously described daily increments, we hypothesized that they were a basis for age estimation.

Observations of sagittae sections also revealed translucent and opaque zones. Slow-growth zones (opaque zones) were more compact with narrower increments than translucent zones. Increments observed within the opaque zones were about 0.2 μm wide, whereas the increments in the translucent zones were 0.5-1.0 μm wide. We hypothesized that the number of increments between the proposed annuli (opaque zones) should approximate the number of days in the faster growth periods of the year. Counts of increments in the same section with both SEM and compound microscopy (n = 21) were performed from the core to the second slow-growth zone and significant differences between counts were not detected (Student's t-test, $P = 0.05$).

Since both methods produced similar counts, the readings on the compound microscope were used to approximate the number of increments between annuli. The first opaque zone occurred after the formation of an average of 187 ± 98 ($\bar{x}$ ± SD) fine increments (n = 20). Between the first and second annulus we counted 250 ± 68 increments (n = 15). We were unable to consistently discern fine increments after the second annulus. However, the distance between the opaque zones (observed as dark bands that spanned the width of the section, Fig. 4) beyond the third annulus was proportionately the same in larger fish.

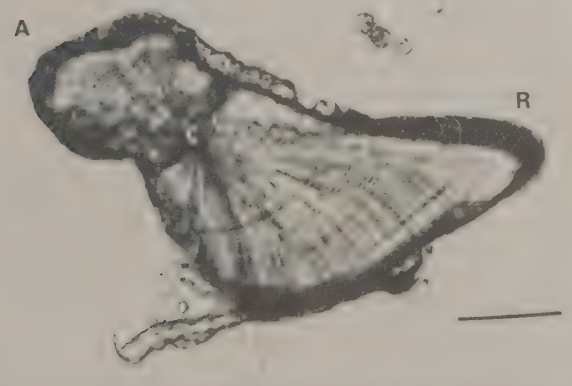

Figure 2.—Light micrograph showing increments in a transverse section of a postlarval swordfish (90.5mm) sagitta (C = core, R = rostrum, A = antirostrum, (bar = 0.05 mm).

Figure 3—Scanning electron micrograph of internal increments along the lateral edge of the rostrum in a transverse section of the sagitta of a 49 kg male swordfish (bar = 10μm).

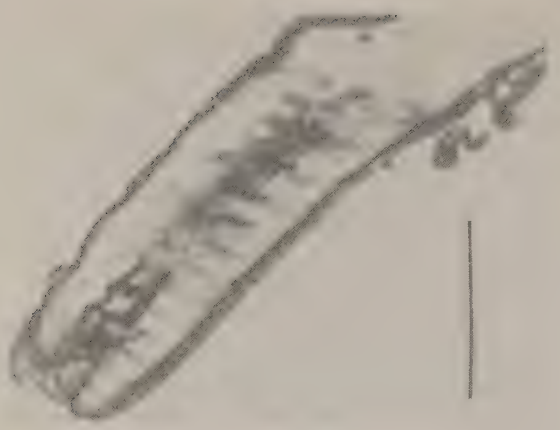

Figure 4.—Light micrograph of annuli (dark zones) present along the rostrum of a 242 kg. (533 lb) female Atlantic swordfish (bar = 0.5 mm).

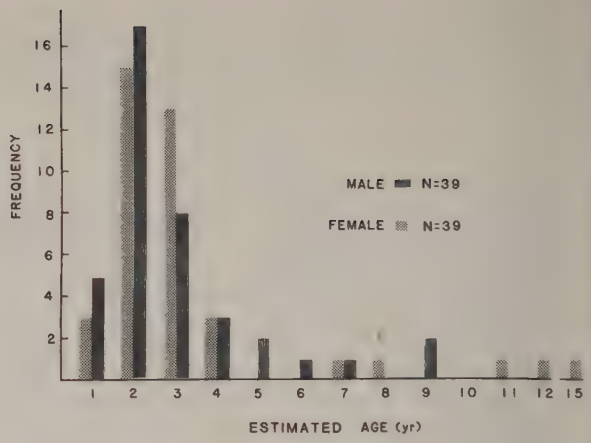

Figure 6.—Frequency histogram of estimated ages 1-15 for male and female Atlantic swordfish collected between Cape Hatteras, N.C., and Florida, 1980-81.

Examination of cross sections of the second anal spine further supported our hypothesis that the opaque zones are annuli in swordfish otoliths. Age estimation using spine and sagittae sections of 45 swordfish gave similar results (89% agreement) with no statistical difference between the counts of these hardparts ($n = 45$, $P = 0.05$, paired t-test). There was also excellent agreement between the age estimates (91%) made by our laboratory using sagittae and those made by S. A. Berkeley (footnote 3) using the spines from the same fish ($n = 11$).

Age estimates of Atlantic swordfish were based on annuli observed in sectioned sagittae, given the assumption that if increments are formed daily, then opaque zones represent annuli. Age estimates ranged from 50 d to 15 yr, with a maximum age of 9 yr for males and 15 yr for females. Males and females appeared to have different growth rates based on the relationship: $L_t = 110.72 \exp [0.0539(t)]$ ($r = 0.80$) for males, and $L_t = 115.64 \exp [0.0609(t)]$ ($r = 0.58$) for females (Fig. 5), as there was a statistical difference between the slopes in these regressions ($P \leq 0.05$). The frequency distribution of each age class was determined and indicated that fish with estimated ages 2 and 3 dominated the commercial catch during sampling periods (Fig. 6).

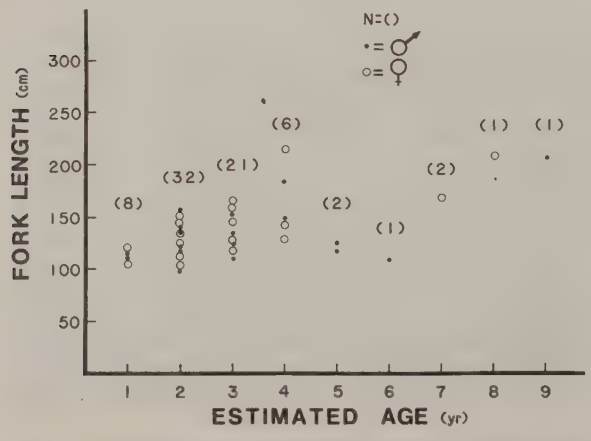

Figure 5.—The relationship between fork length and estimated age of Atlantic swordfish (N = 78).

DISCUSSION

Otoliths of the broadbill swordfish have a general morphology that is now recognized as characteristic of the billfishes. Saddle or cup-shaped sagittae, wing-like lapilli, and ovate asterisci are common to swordfish and have been reported for Atlantic blue marlin (Radtke et al. 1982) and sailfish (Radtke and Dean 1981) and observed in Pacific blue marlin; striped marlin, *Tetrapturus audax*; shortbill spearfish, *Tetrapturus angustirostris*; white marlin, *Tetrapturus albidus*; longbill spearfish, *Tetrapturus pfluegeri*; and black marlin, *Makaira indica* (Radtke 1983; Wilson and Dean[5]). However the sagittae of Atlantic swordfish did have specific morphological differences that readily differentiate the Xiphidae from the Istiophoridae. The extended rostrum of swordfish sagittae, observed even in the youngest fish sampled, was not reported for Atlantic blue marlin sagittae (Radtke et al. 1982) or Atlantic sailfish (Radtke and Dean 1981). Along the mediodorsal edge of the rostrum of sagittae, ridges were proposed as sources of age estimation in Atlantic sailfish (Radtke and Dean 1981) and Atlantic blue marlin (Radtke et al. 1982), Pacific blue marlin, black marlin, striped marlin, and white marlin (Radtke 1983), but we did not readily observe them on the rostrum of swordfish.

The slopes of the length-weight relationship of Atlantic swordfish reported herein were similar to those of Guitart Manday (1964), who reported that swordfish between estimated age class 4 (114 cm LJFL) and 8 (204 cm LJFL) had a relationship, $W = 0.468 \times 10^{-6} L^{3.64}$, when sexes were pooled. Pooling our data and limiting the regression to individuals between 114 and 204 cm (LJFL) produced the relationship $W = 0.775 \times 10^{-6} L^{3.56}$. The slopes of both of these relationships had higher values than the range of 2.6-3.1 reported by Beckett (1974). These results would indicate that the Canadian swordfish are much longer and/or thinner than the South Atlantic fish, perhaps because of migration, or they are different stocks (Radtke and Hurley 1983).

[5]Wilson, C. A., and J. M. Dean. 1981. Age and growth of istiophoridae from otoliths. Unpubl. manuscr. Belle W. Baruch Institute for Marine Biology and Coastal Research, Department of Biology and the Marine Science Program, University of South Carolina, Columbia, SC 29208.

In the process of analyzing the data, we observed that some small fish had sagittae which were heavier than those from large fish. For example, a 15 kg fish (male) had a sagitta that weighed twice as much as the sagitta from a 75 kg fish (male). It has been proposed that otolith dimensions and weight are proportional to age and good indicators of growth in fishes (Templeman and Squires 1956; Fitch and Brownell 1968; Frost and Lowry 1981). Based on this observation, the larger (heavier) saggita observed in our sample should be from an older fish. Since small fish have otoliths as heavy as larger fish of the same sex, it would seem likely that swordfish have highly variable individual growth rates. This observation is similar to that made by Berkeley and Houde (1983) for Florida swordfish. The highly variable results indicate that fish lengths and weights are not good measures for estimation of age of swordfish (particularly past the first couple of years), using length frequency analysis.

In our age analysis of swordfish, we used presumed daily increments to support the presence of annual increments in the sagittae. We recognize that the number of increments observed between the first and second annulus and the second and third annulus did not equal 365. However, this may have been due to our inability to enumerate increments within the opaque zones. We estimated the number of increments in the opaque zones based on increment width and opaque zone width. Combining estimated increment number in the opaque zone with increments actually enumerated between opaque zones produced numbers near 360 and, although this is an approximation, we believe it is indicative of the overall trend.

Predictions of length at a given age were based on combined male and female data, which produced the equation $L_t = 112.23 \exp [0.0601(t)]$, $r = 0.72$. This enabled us to compare our age data with previously published estimates (Table 2). Our analysis produced length at age estimates slightly higher (up to estimated age 2) but similar to those of Berkeley and Houde (1980, 1983), which differ appreciably from predicted lengths at age from other studies. Length-at-age estimates of swordfish by Ovchinnikov (1970), Arata (1954), and Beckett (1974) performed by modal analysis and/or limited tag and recapture data are lower at size than our age estimates and lengths or those of Berkeley and Houde (1980) at similar weights or lengths. In addition, estimates of length-at-age from North Atlantic swordfish reported by Radtke and Hurley (1983) based on otoliths were also lower at size than our study. There seems to be an obvious need for additional work in this area. We attemped to fit the von Bertalanffy growth model to our data. However, the models produced a very low r^2 (0.12), probably due to the lack of adequate numbers of large and small fish.

A frequency histogram (Fig. 6) shows that the commercial catch of our sample set was dominated by young fish (2-3 yr). This pattern is consistent with the data of Berkeley and Houde (1983).

Age estimation of swordfish is still in its developmental stages. It is only now that the necessary tools such as increased sample size, hardpart availability, and laboratory technologies are available to examine the problem of age estimation in this species and the Istiophoridae. Large numbers of swordfish otoliths, fin spines, and morphometric data are still being collected by our laboratory, as are other ecological data. The verification of two methods of age estimation (otoliths and fin spines) gives us confidence that these hardparts, traditionally used for age estimation and validated for other species of fish, are recording the same environmental stimuli. The results suggest that they compliment one another as a source of age information. The counts of presumed daily growth increments on juveniles, and counts of the areas between slow-growth zones on young swordfish, support the assumption of annual increment deposition in swordfish sagittae. Thus, it is now possible to acquire data sets for age estimates of large pelagic predatory fish in the commercial fishery. This is critical information for those concerned with population dynamics of fishery resources and will enable them to formulate management plans with accurate age estimates.

Table 2. – Estimated lengths-at-age of swordfish from different studies (lower jaw fork length = LJFL, orbit fork length = OL, and total length = TL).

Estimated age	Arata (1954) (OL)	Berkeley and Houde (1980) (LJFL)	Guitart-Manday (1964) (TL)	Ovchinnikov (1970) (OL)	Wilson and Dean (text footnote 4) (LJFL)
1	50-60	100		65	120
2	80-90	118	160	90	127
3	100-120	135		110	135
4		151		140	143
5		166		150	152
6		179		170	161
7		190		200	171
8		201		210	182
9					192
10					205

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SHARKS

Techniques for Enhancing Vertebral Bands in Age Estimation of California Elasmobranchs

GREGOR M. CAILLIET, LINDA K. MARTIN, DAVID KUSHER, PATRICIA WOLF, and BRUCE A. WELDEN[1]

ABSTRACT

Vertebrae from 1,152 elasmobranchs representing 22 species were collected between 1979 and 1981 to assess methods of enhancing incremental growth bands for age estimation. Thus far, we have tested methods previously reported in the literature, and have developed new procedures to enhance growth increments on 684 individuals of 14 species of elasmobranchs. Silver nitrate impregnation, X-radiography, and cedarwood oil clearing were the most successful techniques. Less effective were alizarin red staining, paraffin impregnation, alcohol immersion, and formic acid etching. Methods for preparing vertebrae and enhancing and counting growth increments are presented, and the problems associated with interpreting the annual nature of such counts are discussed.

INTRODUCTION

Little is known about the age, growth, and reproduction of elasmobranchs because many species are difficult to sample, are of relatively large size, are highly mobile, exhibit seasonality, and are of minor commercial value. In addition, many of the conventional age determination methods used for bony fishes are not applicable to elasmobranchs because elasmobranchs lack calcareous otoliths and other skeletal hardparts. In California, the commercial exploitation of elasmobranchs has been rapidly increasing, making information about their life histories essential for understanding and managing their populations.

Several methods of age determination have been developed for elasmobranchs. Length frequency analysis has been used by Templeman (1944), Olsen (1954), Aasen (1963), Parker and Stott (1965), Johnson and Horton (1972), Sage et al. (1972), and Edwards (1980). Often, this kind of analysis is coupled with tag-recapture studies (Steven 1936; Kauffman 1955; Babel 1967; Davies and Joubert 1967; Kato and Carvallo 1967; Wass 1973; Holden 1974; and Grant et al. 1979). These two approaches are limited due to the slow growth rates exhibited by elasmobranchs, and sampling difficulties. Moss (1972) used tooth replacement rates to estimate growth rates, but this technique provides only rough estimates, as the tooth replacement rate varies among individuals. Using the developmental state of secondary sex characters, Johnson and Horton (1972) could only categorize fish into "young, immature, and adult age groups." Embryonic growth rates have also been used to generate growth curves by extrapolation (Ketchen 1972; Holden 1974; Francis 1981), but "it is not a substitute for growth rate analysis based on age determination, and should only be used as an interim measure" (Francis 1981). Dorsal spines have been examined by Kaganovskaia (1933), Templeman (1944), Bonham et al. (1949), Aasen (1961), Holden and Meadows (1962), and Ketchen (1975), and were found to have incremental zones (see Glossary). Because most elasmobranchs do not have spines, this technique has limited applicability.

Growth zones deposited in vertebral centra are promising tools for age determination of elasmobranchs. Ridewood (1921) first described these zones in his review of calcification processes, and Urist (1961) and Applegate (1967) provided further morphological evidence that these zones were common among sharks and rays. Haskell (1949) first suggested that these zones could be useful in age determination studies. Several authors then developed and used various techniques to enhance these zones in several species of elasmobranchs, including alcohol immersion (Richards et al. 1963), xylene impregnation (Daiber 1960), alizarin red (LaMarca 1966), histology (Ishiyama 1951), silver nitrate impregnation (Haskell 1949; Stevens 1975), X-radiography (Urist 1961; Aasen 1963; Applegate 1967), and X-ray spectrometry (Jones and Geen 1977).

Various authors have postulated that these growth zones are deposited annually. Ishiyama (1951), working with the Japanese black skate, *Raja fusca*, tentatively concluded that the alternating zones were laid down in winter. Daiber (1960) and Richards et al. (1963) found that their growth data for two other species of skate fit the von Bertalanffy (1938) growth equation, and concluded that their zones were probably annual. Stevens (1975) estimated age of blue sharks, *Prionace glauca*, using silver nitrate, and found that his data correlated well with Aasen's (1966) length-frequency data. Several authors have used tetracycline to mark bony structures in fishes (Weber and Ridgway 1962; Simkiss 1974), and recent studies using tetracycline on elasmobranchs (Holden and Vince 1973; Graber and Stout 1983) support annual zone formation in their centra. Finally Jones and Geen (1977) used an energy-dispersive X-ray spectrometric system to detect peaks of the elements calcium and phosphorus, which they concluded were deposited annually in the centra of the spiny dogfish, *Squalus acanthias*.

One of the answers to Holden's (1977) plea for "establishing acceptable techniques" in age determination of elasmobranchs may lie in the concentric zones found in their centra. Because the amount and pattern of calcification may vary considerably among species (Ridewood 1921; Haskell 1949; Urist 1961; LaMarca 1966; Applegate 1967), a comprehensive review and evaluation of age determination methodology are needed. Since 1979, we have attempted to determine the most effective methods of enhancing the visibility of these zones in centra

[1]Moss Landing Marine Laboratories, Moss Landing, CA 95039-0223.

from California elasmobranchs. We have experimented with cleaning, slicing, and grinding procedures to prepare vertebrae for subsequent age determination, and have used numerous enhancement methods to expose the zones for counting. We present here our evaluation of several enhancement methods, and discuss counting procedures and problems associated with interpreting the periodicity of zone deposition.

COLLECTING, PROCESSING, AND PREPARATION

We have utilized several sources to obtain specimens for testing the various age determination techniques. The catches at several shark derbies conducted in Elkhorn Slough, Calif., (Herald et al. 1960) provided us with samples of the leopard shark, *Triakis semifasciata*, brown smoothhound, *Mustelus henlei*, and bat ray, *Myliobatis californica*. We have also sampled with trawls and gill nets to obtain other local coastal species such as the blue shark, gray smoothhound, *Mustelus californicus*, and spiny dogfish. Commercially important elasmobranchs were obtained in central and southern California by subsampling the commercial gill net, trammel net, and trawl fishing fleet catches. This produced specimens of the common thresher, *Alopias vulpinus*, shortfin mako, *Isurus oxyrinchus*, soupfin, *Galeorhinus zyopterus*, and Pacific angel, *Squatina californica*, sharks, in addition to the longnose, *Raja rhina*, and big, *R. binoculata*, skates. Specimens of the basking shark, *Cetorhinus maximus*, and the great white shark, *Carcharodon carcharias*, were obtained incidental to commercial gill net catches (Table 1). We have collected 1,152 specimens representing 22 species.

For each individual specimen collected, measurements were taken, the reproductive tract examined, and approximately 12 vertebrae removed, usually from below the origin of the dorsal fin, and frozen in plastic bags. Measurements included length (total, precaudal, and distance between dorsal fin origins), girth, and weight. To assess reproductive condition in males, the configuration of the vas deferens and the condition, size, and development of the claspers were noted. For some species, sperm smears were made and microscopically examined to verify presence of mature sperm (Pratt 1979). For females, the number and size of eggs in the ovaries were recorded; the embryos, if present, measured and sexed; oviducal gland and oviduct dimensions recorded; and presence or absence of uterine scars noted. This information was used to determine the size and age at which the different species reach sexual maturity.

Once defrosted, the neural and haemal arches and connective tissue must be removed from each vertebra to expose the centrum surfaces which contain the zones. This was accomplished using one of several techniques, depending upon the species. For Pacific angel, shortfin mako, common thresher, blue, and great white sharks, a 5-min soak in distilled water followed by air drying effectively allowed the connective tissue to be peeled away from the centrum. Soaking in bleach was more effective for removing connective tissue from leopard shark, gray and brown smoothhounds, spiny dogfish, bat ray, big skate, and longnose skate centra. Longer soaking time in bleach was needed for larger centra, and immersion intervals ranged from 5 to 30 min. Finally, the centrum was rinsed well in tap water. Soaking in enzyme detergent solutions and subjecting the centrum to ultrasonic cleaning procedures did not significantly enhance the cleaning that had already resulted from bleach immersion.

TECHNIQUES FOR ENHANCING BANDS IN VERTEBRAL CENTRA

We have found in nearly every elasmobranch centrum examined that the zones are the result of two kinds of concentric marks (Fig. 1). We define a "ring" as the narrowest kind of concentric mark observed, and use the term "band" to refer to wider concentric marks composed of groups of rings. Therefore, we interpret the wider bands to contain widely spaced rings, while narrow bands have rings that are more tightly spaced.

Cleaned centra were often sectioned, either along a transverse or longitudinal plane (Fig. 1b) to prepare them for three band-enhancement techniques. This sectioning was especially

Table 1.—Summary of collection and processing activities from 1979 to 1981 showing the number of each species of elasmobranch collected and aged, and the relative effectiveness of three techniques for clarifying bands in vertebral centra. These techniques were evaluated as those that provided repeatable counts (+), did not provide repeatable counts (−), or were not tried (?).

Common name	Scientific name	Number sampled	Number age estimated	Technique		
				Silver nitrate	X-radiography	Oil clearing
Bat ray	*Myliobatis californica*	191	191	−	+	+
Leopard shark	*Triakis semifasciata*	136	131	+	−	?
Brown smoothhound	*Mustelus henlei*	50	50	+	+	?
Gray smoothhound	*Mustelus californicus*	38	38	+	+	?
Common thresher	*Alopias vulpinus*	57	57	+	+	+
Shortfin mako	*Isurus oxyrinchus*	23	23	+	+	+
Blue shark	*Prionace glauca*	26	26	+	+	+
Pacific angel shark	*Squatina californica*	56	41	+	+	?
Soupfin shark	*Galeorhinus zyopterus*	70	0	+	?	?
Longnose skate	*Raja rhina*	196	35	−	−	+
Big skate	*Raja binoculata*	188	50	−	−	+
Spiny dogfish	*Squalus acanthias*	70	40	−	−	?
Basking shark	*Cetorhinus maximus*	2	1	+	+	+
Great white shark	*Carcharodon carcharias*	9	1	+	+	?
		1,112	684			
8 additional species		40	0			
Total		1,152	684			

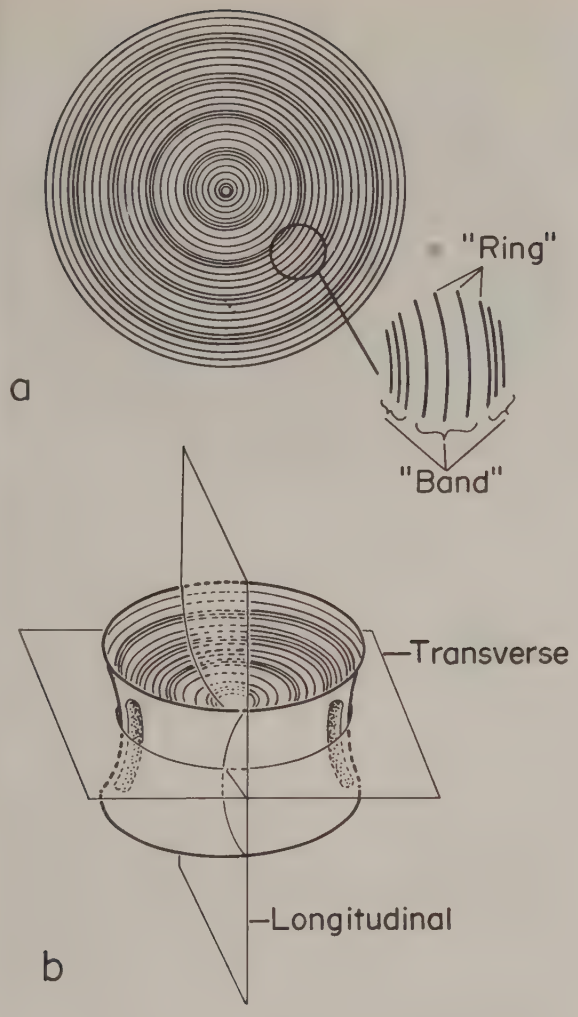

Figure 1.—Diagram of typical elasmobranch centrum showing (a) bands made of fine "rings"; and (b) the two sectioning planes used.

needed for centra that had relatively deep cones (Fig. 2), as opposed to those that were flat or disklike along the longitudinal plane (Figs. 3, 4). Large vertebrae secured in a vise were cut in half with a small circular saw attachment on a jeweler's drill. For smaller specimens, half of the centra was ground away using aluminum oxide wheel points and fine sandpaper attachments for the same tool. Transverse sectioning prevented bands on these opposing halves from obscuring each other when observed after further preparation (Fig. 1), and longitudinal sectioning enhanced the finer bands laid down at the centrum edge.

Three techniques were consistently useful for enhancing bands in centra, while several other techniques have either proven ineffective or have not yet been evaluated.

The first technique was adopted by Stevens (1975) to enhance bands in blue shark centra. Calcium salts in the centrum are replaced with silver, providing distinct silver-impregnated bands which become quite dark after illumination under ultraviolet light. The narrow bands have more tightly spaced rings, and therefore appear darker than the broad bands (Figs. 2, 3a).

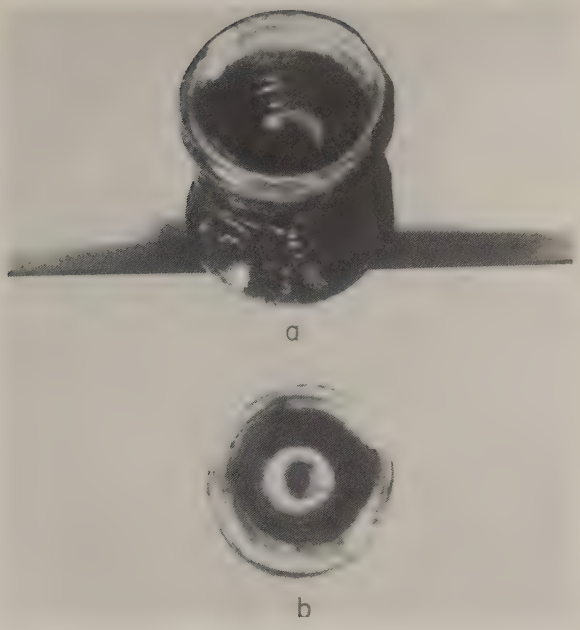

Figure 2.—Oblique (a); and anterior (b) views of the same vertebral centrum taken from a 114 cm TL mature female leopard shark and stained with silver nitrate. This centrum was determined to have nine bands.

An advantage of this technique is that vertebrae preserved in 70% alcohol, as well as fresh specimens, may be used. It was necessary to further modify Stevens' procedures. To assure the chemical substitution of silver for calcium, all connective tissue was removed from the centrum by one of the previous cleaning methods. To remove any traces of bleach and to etch its surface, the centrum was soaked in concentrated (88%) formic acid for 2-4 min. The centrum was then soaked in distilled water for approximately 15 min. Then it was placed in a 1% silver nitrate solution and immediately placed in a chamber where it was illuminated by an ultraviolet light source (GE[2] F15T8-BLB) for 3-15 min, depending upon the species tested and the size of the centrum. The centrum was then rinsed in distilled water to remove excess silver nitrate.

Usually, a dissecting microscope with reflected illumination focused laterally on the centrum was used to count bands. Several centra (3-5) from each specimen were stained and counted for replicate analysis. After these counts were made on the newly stained centra, they were soaked in a 5% sodium thiosulfate solution for 2-3 min. This procedure removes excess silver and fixes the chemical substitution. Because fixation often eradicates the very narrow rings, counts should occur before fixation if counts of these rings are desired. Band counts were made before and after fixation. The final step was storage in 70% isopropyl alcohol.

The second technique involved taking X-radiographs of half-centra as prepared above. We have used a Hewlett-Packard Faxitron Series X-Ray System (Model No. 43805N) with Kodak Industrex M film (Readypack M-2), as suggested by Miller and Tucker (1979). X-radiographs of bat ray centra were viewed

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

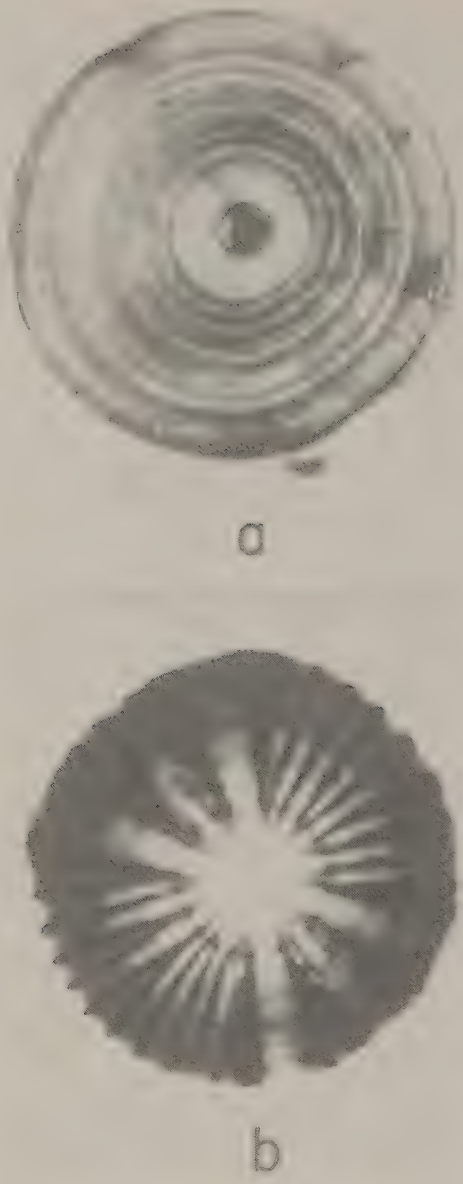

Figure 3.—Two vertebral centra from the same specimen of shortfin mako shark with bands enhanced using (a) the silver nitrate technique; and (b) the X-radiography method. The centrum diameter from this 211 cm TL immature female was 26 mm, and there were six or seven bands, as determined by these two techniques.

through a dissecting microscope with a combination of reflected and transmitted light. X-radiographs of centra from Pacific angel, common thresher, blue, basking, great white, shortfin mako, and both species of smoothhound sharks were viewed through a compound or dissecting microscope using transmitted light from below. In X-radiographs, the narrow bands appear white, while the broad bands, which have less tightly spaced rings, appear darker (Figs. 3b, 4).

The third technique involved applying cedarwood oil to the face of each centrum to increase the clarity of bands by eliminating superficial irregularities. Frequently, scraping of the

Figure 4.—Radiographs of the vertebral centra of three sizes and developmental stages of Pacific angel shark. (a) is from a 1,110 mm TL adult female, widest centrum diameter 17 mm; (b) is from a 360 mm TL free-living juvenile, centrum diameter 6 mm; (c) is from an unborn, pre-term embryo with yolk sac that measured 225 mm TL and had a 3 mm centrum diameter.

centrum face with a scalpel enhanced the clarity of the finer bands. The bands of the centrum thus prepared were viewed under a dissecting microscope using a fiber optics light transmitted both vertically and horizontally over a dark background. Using this approach, the bands that are composed of more tightly spaced rings (narrow bands) appear darker than those with less tightly spaced rings (broad bands).

Attempts at using several other published techniques were less successful at enhancing bands in centra for the species we examined. Following Daiber's (1960) technique for the clearnose skate, *Raja eglanteria*, centra from both the longnose and big skates were soaked in Formalin, cleared of connective tissue, and placed in 95% isopropyl and then in absolute isopropyl alcohol. After immersion in xylene, the centra were heated in paraffin at 60 °C and returned to xylene. Most of the centra prepared in this manner were only partially cleared, and bands were unclear. In testing the technique of Richards et al. (1963), centra from the same two skate species were either cleaned in sodium hydroxide or scraped, placed first in 70% isopropyl alcohol and then transferred to 100% alcohol. Bands especially on the outer portions of the centrum, were unclear, irrespective of cleaning technique. Using a third method, originally used by LaMarca (1966) on the sand tiger shark, *Odontaspis taurus*, a small number of centra from longnose skates were

cleared in sodium hydroxide, stained in a saturated solution of alizarin red S in sodium hydroxide, and differentiated in 3% hydrogen peroxide. Although success in enhancing growth bands on centra was variable and ring contrast was moderate, further attempts with this technique are warranted and may yield better results. Smith (1980) used a technique on the cownose ray, *Rhinoptera bonasus*, in which vertebrae were stored in alcohol, cleaned, air-dried, and sectioned longitudinally. After the hourglass-shaped face was polished, the centra were heated at 200 °C for 2-3 min. This technique did not enhance bands in centra of the Pacific bat ray. Finally, our attempt to use Stirling's (1969) method originally designed for delineating rings in pinniped teeth, which involves etching for 24 h with formic acid and Formalin, did not noticeably enhance bands in leopard shark centra.

Undoubtedly, there are many other procedures that may prove useful in aiding researchers to enhance bands in elasmobranch vertebrae. One, which we have not yet had the facilities to pursue, is the use of X-ray or electron microprobe spectrometry to measure such elements as calcium and phosphorus, which are more concentrated during certain seasons than in others (Jones and Geen 1977; Casselman 1983). This method is somewhat expensive and time-consuming, but may provide valuable information for comparing results with other, more practical techniques.

EFFECTIVENESS OF TECHNIQUES

Procedures for counting bands in centra were standardized to ensure consistent and objective evaluation of the various techniques among species and among researchers. For all species and techniques, at least two observers made replicate and independent counts of both narrow and broad bands in each centrum. If these initial counts varied by more than one set of bands and additional readings did not result in agreement, the centrum was not used for age analysis. In general, there was good agreement among observers, with $< 10\%$ of the counts disagreeing by more than one band pair. As is common in many age determination studies, there was greater agreement of band counts from younger age classes. Each technique was considered to be effective if it followed these criteria and produced consistent and repeatable band counts.

We were able to delineate and count bands on centra of all species tested using at least one of the three enhancement techniques (Table 1). Using our modification of the Stevens' (1975) silver nitrate staining technique, bands were clearly discernible in 10 of the 14 species tested, and examples of centra treated with silver nitrate are shown in Figures 2 and 3b. This technique did not produce repeatable band counts in species that had centra with a poorly differentiated calcification pattern, poor calcification, or only narrow and tightly spaced bands. Schwartz (1983) also used this method to estimate age of dusky, *Carcharhinus obscurus*, and scalloped hammerhead, *Sphyrna lewini*, sharks off North Carolina.

Distinct bands were discernible in 9 of the 13 species tested using X-radiography (Table 1; Figs. 3b, 4). The X-ray technique was not successful in providing repeatable counts with centra of the spiny dogfish, leopard shark, and the two skates tested, apparently due to diffuse calcification patterns, and to radiating structural components in these vertebrae that interfere with the clarity of the bands (Ridewood 1921). For species with narrow, elongate (deep-coned) centra, such as gray and brown smoothhounds, it was necessary to section these centra longitudinally so that the bands could be clearly observed in radiographs.

The oil-clearing technique worked well on all seven species tested (Table 1). Because transmitted light was used with this technique, it detected differences in optical density through the centrum, and was not simply an examination of surface topography.

In general, those specimens with ages estimated by any two of these techniques (Table 1) agreed with each other. For example, 92% of the 130 bat rays aged by both X-radiography and oil clearing were placed in the same age class or differed by only 1 yr, with most of the disagreements occurring in the oldest fish (Martin 1982). Similar results for smaller samples were found for the common thresher, shortfin mako, blue, and basking sharks. In species in which band counts were compared using both X-radiography and silver nitrate, counts were also extremely similar. For example, 90% of the 31 gray and 82% of the 45 brown smoothhounds aged by these two techniques were placed in the same age class or differed by only 1 yr (Kusher[3]). Centra prepared by these two techniques for the shortfin mako also produced quite similar results, with the example shown in Figure 3 producing counts of 6 or 7 bands for the same individual. Similar results from smaller samples were found for the common thresher, shortfin mako, blue, Pacific angel, basking, and great white sharks.

INTERPRETATION OF BAND COUNTS

Once a sufficient number of centra has been used to estimate age by counting bands, growth curves can be constructed using several models that have been reviewed extensively by Ricker (1979). To confidently interpret the meaning of band counts in elasmobranch centra, it must be demonstrated that band formation provides a continuous record of growth, and the count of bands represents known intervals of time.

The assumption that centra are good indicators of age is supported by three lines of evidence. First, in elasmobranchs, growth of the calcified cartilagenous skeleton occurs by a one-way process of deposition, and there is no indication of internal remodeling or resorption (Ridewood 1921; Urist 1961; Applegate 1967; Simkiss 1974). Second, increased body sizes are accompanied by increases in centrum diameters (Stevens 1975), because the centrum must grow in order to accommodate new growth bands. Third, because the banding pattern visible in X-rays (Gosline 1948) and in the other two techniques occurs as a result of density differences in subject matter, it is likely that the difference between the high and low density bands is due to differences in mineralization occurring during different growth phases. As suggested by Ishiyama (1951) and Jones and Geen (1977), the pattern of mineralization may be strongly influenced by seasonal environmental changes which may, in turn, affect growth rates. The presence of a heavily mineralized peripheral band in the majority of young bat rays and leopard sharks collected during the summer months, and a lightly mineralized band in winter-caught specimens, offer further sup-

[3]Kusher, D. Age, growth and reproduction of the leopard shark, *Triakis semifasciata*, gray smoothhound, *Mustelus californicus*, and brown smoothhound, *Mustelus henlei*. M.A. Thesis in prep. Moss Landing Marine Laboratories, P.O. Box 223, Moss Landing, CA 95039.

port for the assumption of faster growth in summer and slower growth in winter (Martin 1982; Kusher footnote 3).

It is generally accepted, but still an assumption, that the growth bands found in the hardparts of temperate teleosts are of an annual nature (Williams and Bedford 1974; Holden 1977), but this has not been adequately validated for bands in elasmobranch centra. Numerous authors studying elasmobranch growth have postulated or assumed annual band formation in centra (Aasen 1963; Richards et al. 1963; Taylor and Holden 1964; Stevens 1975), but conclusive age validation studies using such techniques as tag-recapture and tetracycline injection have only been tested on several elasmobranch species (Holden and Vince 1973; Holden 1974; Gruber and Stout 1983).

For several Pacific species, we have produced growth curves and have used several approaches to assess whether their bands are annual. Often, different verification methods produce inconclusive or conflicting results, and seldom can the majority of them be applied to one species (Brothers 1983). These methods can be characterized as those which: 1) Require random sampling of numerous individuals over time while monitoring changes in their size classes and centrum characteristics, 2) compare vital growth model parameters with known size information, such as size at birth (L_0) and maximum observed size (L_∞), and 3) measure individual growth using tagged fish from field recaptures or in laboratory growth experiments. This last approach can also include centrum band-marking techniques.

The first verification approach is one of the most commonly used, because some species are relatively easy to sample. We have compiled size-frequency histograms for bat rays, leopard sharks, gray and brown smoothhounds, and blue sharks, and have compared the mean sizes of the first several modes with the growth increments predicted by growth curves generated from band counts with good agreement (Kusher footnote 3). Also, the changes in mean size of young-of-the-year leopard sharks collected monthly in Elkhorn Slough corresponded well with early growth as determined from band counts, thus verifying that band counts can be used as indicators of early ages (Kusher footnote 3).

In newborn individuals, the number of bands can be used as an indication of gestation period, assuming the bands are laid down over some regular interval prior to birth. Newborn leopard sharks lack a complete set of bands, one dark and the other lighter, using silver nitrate impregnation, suggesting that these band pairs may be laid down at least annually, and, if so, that their gestation period is a year or less (Kusher footnote 3). Because leopard sharks in central California are born in spring and early summer, and the center of their centrum is dark when using silver nitrate stain, it is presumed that the dark band represents summer growth, while the lighter band was formed during winter months. Angel shark embryos, on the other hand, have fewer than five bands (Fig. 4c), and newborn individuals have between 6 and 7 bands (Fig. 4) when viewed through X-radiographs, indicating that these bands are laid down in less than annual fashion, mark physiological events, or that these fish have a very long gestation period. Ridewood (1921) depicted a similar number of bands in the vertebra of a ripe embryo of the angel shark, *Squatina squatina*, thus indicating that band formation may be similar among all species in this genus.

The width and density of the centrum edge can also be used to indicate the temporal periodicity of band formation. Because it is difficult without histological preparation to delineate the centrum edge in detail, and because the edge is often irregular in width, we have not yet used the width of the peripheral band to evaluate this approach in elasmobranchs we have studied. However, by categorizing peripheral bands as dark or light when treated with silver nitrate, and comparing the proportion of both summer- and winter-caught specimens with each of these categories of peripheral bands, we have been successful at interpreting seasonality of band formation. For example, most bat ray and leopard shark centra collected in Elkhorn Slough during summer months had dark peripheral bands (Martin 1982; Kusher footnote 3), thus providing indirect evidence that their bands are formed during the summer. This is also supported by the prevalence, during winter months, of the lighter bands at the edges.

Another example of the first approach is to use histological techniques to identify "growing zones" or "peripheral calcification" areas in centrum sections (Ridewood 1921; Urist 1961; Applegate 1967; Andrew and Hickman 1974) in younger individuals collected over time. So far, we have only experimented with this approach, using vertebral centra from a blue shark collected during the summer of 1982. Longitudinal sections 15 μm thick were made using a microtome on centra decalcified with dilute (4%) nitric acid or Cal-Ex, and stained with haemotoxylin and eosin. The decalcification procedure caused some shrinking, so centra should be preserved in Formalin first if measurements are desired, because this procedure reduces the shrinking. Two cell types were apparent in these bow-tie-shaped sections, especially the outside edge. The peripheral cells were narrow or squamous in appearance, while the more proximal adjacent cells were square or cuboidal. Alternating squamous and cuboidal layers continued toward the center of the centrum, but were less distinct. The blue shark examined produced superficial band pair counts totalling six or seven using silver nitrate staining, which agreed with counts made using histological sections. We feel, therefore, that this approach appears promising, and we hope to apply it to centra of other species of elasmobranchs.

The second approach useful in age verification is to compare growth model parameters with known size information, such as size at birth and maximum observed size. Even though growth models may not perfectly fit a given set of size and age estimate data, and information on size at birth and maximum observed size may not be good estimates of mean values, this approach does produce a rough approximation. We have now used this approach comparing vital parameters of the von Bertalanffy (1938) growth equation to size information from catch records for seven species, and results indicated relatively close agreement. Three of these species (the common thresher, shortfin mako, and blue shark) are reported in Cailliet et al. (1983), while three others (the bat ray, *Myliobatis californica*, and two species of smoothhound, *Mustelus henlei* and *M. californica*) are reported elsewhere (Martin 1982; Kusher footnote 3).

As an example, we report here on the leopard shark, which has an estimated size at birth from many measurements of newborn individuals in central California (200-220 mm TL), which closely corresponds to the length at which the von Bertalanffy curve, based on 130 aged specimens, intersects the ordinate (Kusher footnote 3). In addition, maximum reported size from our catch records and from Miller and Lea (1972) is only 13% higher than the asymptotic length derived for females (Kusher footnote 3).

Although these preliminary results must be tempered by small sample size, and the unsubstantiated assumption that one set of bands is equivalent to 1 yr, our method of counting bands appears to follow the von Bertalanffy growth model for the size range of leopard sharks we examined. Holden (1974) also found that this approach supported estimates of age for several species of skates that he studied.

The third approach, which can be used in both the field and the laboratory, is to monitor the change in body size of tagged individuals over known periods of time. There are problems associated with this approach, but it generates information with which to evaluate growth curves. In the field, difficulties arise in collecting sufficient numbers of animals, making accurate measurements, tagging them without harming them or inhibiting their natural growth rates, and finally, recapturing them after a sufficient period of time has elapsed during which growth can be measured. We have been able to successfully use this approach on leopard sharks tagged by us in Elkhorn Slough, and tagged in San Francisco Bay (Smith[4]). We have plotted lengths and ages (based upon number of bands counted on centra at recapture) on the growth curve and compared them with the size at time of first capture. The slope and position of changes in size have been quite helpful in evaluating our growth curve.

For leopard sharks, several recaptured fish fit the growth curve closely, but several others did not grow, even over 2 yr (Kusher footnote 3). This indicates that all individuals do not grow exactly as the curve would predict or that tagged fish were poorly measured or did not grow. The size and presumed age of tagged individuals will influence this verification technique considerably, because older fish grow more slowly, and changes in their sizes will be less detectable. Thus, growth rates based on tag recapture data from one size class cannot be used to calculate a growth rate for all sizes or age classes.

Organisms maintained under laboratory conditions can be used similarly. A major disadvantage of laboratory grow-out studies is often that the fish are not maintained under natural conditions, and so may exhibit unnatural growth rates. We have attempted to grow bat rays and leopard sharks at Moss Landing Marine Laboratories and Steinhart Aquarium, Golden Gate Park, San Francisco, Calif., but have had only limited success, because many of our specimens failed to eat sufficient quantities of food, and often failed to grow at all. However, given improvements in the ability to maintain and grow marine organisms, this approach will provide valuable information, especially of a short-term nature, when measuring growth rates.

Internal marks, such as tetracycline, can be used in conjunction with traditional tag-recapture techniques to determine the time sequence of band formation (Holden and Vince 1973; Gruber and Stout 1983; Casselman 1983). This approach entails injecting a fish, either in the laboratory or field, with tetracycline, which is deposited into areas of calcification. After a known period of time, the fish is recovered and sacrificed, and its centra examined under ultraviolet light for a band of fluorescence. Holden and Vince (1973), Gruber and Stout (1983), and Smith (footnote 4) have used this method successfully on skates, the lemon shark, *Negaprion brevirostris*, and the leopard shark, respectively. However, our attempts with bat rays have not been very successful. The tetracycline did deposit in the peripheral zone of calcification, but it produced a diffuse band too indistinct to serve as a temporal check.

A very promising validation technique involves using radioactive geochronologies to estimate the relative ages of different bands (Goldberg and Bruland 1974; Turekian and Cochran 1981; Casselman 1983). This technique, which we are still developing, involves analyzing inner and peripheral bands of vertebral centra for naturally occurring radionuclides with relatively short half-lives. The difference in radionuclide activity levels between bands can be used to estimate their ages, because these radionuclides have constant and known decay rates. This technique has been used successfully on rockfish, *Sebastes diploproa*, otoliths (Bennett et al. 1982), and to measure growth rates of clams (Turekian et al. 1975, 1979; Turekian and Cochran 1981) and corals (Moore and Krishnaswami 1972; Moore et al. 1973; Dodge and Thompson 1974). Nuclides such as ^{210}Pb (22-yr half-life) and ^{210}Po (138-d half-life) are appropriate for ageing organisms with lifespans up to 100 yr.

Our preliminary analysis of inner and peripheral bands of centra from the common thresher shark indicate that this approach will be successful. The inner band contained 0.04694 ± 0.00420 dpm (disintegrations per minute)/g of ^{210}Po, and the outer band had 0.1082 ± 0.003314 dpm/g (Welden[5]), indicating that sufficient radioactivity and a closed system exist. Thus, provided that ^{210}Pb is present at comparable levels, the time between band formation can be calculated based on the observed ^{210}Po/^{210}Pb ratio.

Elasmobranch age determination requires the use of several validation and verification techniques because of the huge diversity of elasmobranch life histories. For example, methods (applicable to small bottom-dwelling forms) such as tagging, tetracycline marking, and laboratory grow-out studies will be difficult or impossible to apply to large pelagic species (Brothers 1983). A multiple approach is also valuable because the results of several different techniques can be compared for one species. Only with this kind of comprehensive approach will it be possible to confidently state that the bands we have counted provide valid estimates of the age of elasmobranchs.

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[4]S. Smith, Fishery Biologist, Southwest Fisheries Center Tiburon Laboratory, National Marine Fisheries Service, NOAA, 3150 Paradise Drive, Tiburon, CA 94920, pers. commun. 1982.

[5]B. A. Welden. Unpubl. data. Moss Landing Marine Laboratories, P.O. Box 223, Moss Landing, CA 95039.

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SUMMARY PAPER

Shark Ageing Methods and Age Estimation of Scalloped Hammerhead, *Sphyrna lewini*, and Dusky, *Carcharhinus obscurus*, Sharks Based on Vertebral Ring Counts

FRANK J. SCHWARTZ[1]

INTRODUCTION

Ageing of bony fishes, which began as early as 1759 (Hederström 1959), has often been accomplished by counting growth rings on a variety of skeletal structures such as scales, spines, otoliths, head bones, or vertebrae (Menon 1950; Chuganova 1963; Bagenal 1974). Conversely, elasmobranchs are difficult to age as they possess few hard skeletal structures. I review shark ageing methods and present data on scalloped hammerhead, *Sphyrna lewini*, and dusky, *Carcharhinus obscurus*, sharks, captured in North Carolina, where, with remarks on 10 other species, age was estimated based on counts of vertebral rings stained with silver nitrate.

PREVIOUS SHARK AGEING METHODS

Many early attempts to age sharks were conducted by investigators noting size differences for aquarium or experimentally held specimens (Hisaw and Abramowitz 1937; Clark 1963). Other studies assessed length frequency data to separate year classes of tag-recaptured individuals as a means to estimate age (Templeman 1944; Olsen 1954; Aasen 1963; Ketchen 1975; Davies and Joubert 1967; Kato and Carvallo 1967; Wass 1973; Stevens 1975; Grant et al. 1979). Although the accuracy of age interpretations obtained from length frequency histograms has been increased by plotting the data on probability paper (Cassie 1954), or by using computers (Hasselblad 1966), early age estimates of sharks was still an arduous procedure that lacked a direct ageing method, such as ring counts on skeletal hardparts.

Recently Forrester et al. (1972) and Childs et al. (1973) used mercury accumulations in vertebrae to estimate age in elasmobranchs. However, Forrester's et al. (1972) mercury level-length frequency estimates were substantially less than those predicted by Bonham et al. (1949) who studied the same species but used length-frequency analysis. Childs' et al. (1973) mercury level data were likewise inadequate when applied to pup or small-sized sharks (Ketchen 1975).

Tooth replacement of upper and lower teeth in sharks, which are continuously renewed from posterior to anterior, can be related to body growth (James 1953; Strasburg 1963; Applegate 1965; Moss 1967). For example, knowing tooth replacement rate, Moss (1972) estimated maximum body size and age of maturity for the lemon shark, *Negaprion brevirostris*. Other shark ageing methods utilized fin spines (Kaganovskaia 1933; Holden and Meadows 1962; Ketchen 1975), and vertebral ring counts (Ridewood 1921; Aasen 1963; Parker and Stott 1965; LaMarca 1966; Holden 1974; Stevens 1975).

Most previous shark ageing techniques were unvalidated since growth rings in spines or vertebrae, i.e., of spiny dogfish, *Squalus acanthias* (Holden and Meadows 1962), basking shark, *Cetorhinus maximus* (Parker and Stott 1965), and porbeagle, *Lamna nasus* (Aasen 1963), had not been substantiated as annual events. Parker and Stott (1965) examined unstained vertebrae of the basking shark and suggested that two rings were formed annually based on a correlation of a hypothetical asymptote growth curve calculated from size frequency data. However, this information has been criticized by Pauly (1978). Stevens (1975), Holden and Vince (1973), Cailliet, Martin, Harvey, Kusher, and Welden (1983); Cailliet, Martin, Kusher, Wolf, and Welden (1983); Casey et al. (1983); Gruber and Stout (1983); and Pratt and Casey (1983) also counted, often under reflected light, the circuli in the centra of vertebrae and thereby estimated age. Although LaMarca (1966), studying the sand tiger, *Odontaspis taurus*, established that vertebral rings were present, he did not know if these rings were actually calcified or just "a peculiar tinctorial property of the centra." Several vertebrae were decalcified by LaMarca (1966) in 5% nitric acid for 18 h before staining. Instead of being stained, these vertebrae were completely colorless. This suggested that the stained areas were areas of concentrated Ca^{++}. Calcified vertebral rings have also been reported and related to age for: Porbeagle (Stevens 1975); basking shark (Ridewood 1921; Parker and Stott 1965; Springer and Gilbert 1976); eiraku shark, *Galeorhinus japonicus*, (Tanaka et al. 1978); blue shark (Stevens 1975); sandbar shark, *Carcharhinus plumbeus*, (Springer 1960; Wass 1973; Casey et al. 1983); and other species. Ridewood (1921) suggested ring calcification may be a response to physiological demands of the cartilage.

Holden and Vince (1973), using tetracycline as an internal tag, were the first to validate elasmobranch vertebrae ageing methods by establishing that opaque and translucent zones were formed annually in the skate, *Raja clavata*, and could be used as a means to count rings as age markers. Gruber and Stout (1983) also used tetracycline in age studies of the lemon shark. Urist (1961), employing X-radiography, determined that the various densities within shark vertebrae were associated

[1]Institute of Marine Sciences, University of North Carolina, Morehead City, NC 28557.

with Ca^{++} and P^{2} depositions. Jones and Geen (1977), using radiography on the spiny dogfish, also noted that vertebral rings contained high levels of Ca^{++} and slightly lower levels of P^{2}. Others that used X-ray methods to age sharks were Ishiyama (1952), Aasen (1963), Applegate (1967), Cailliet, Martin, Harvey, Kusher, and Welden (1983), and Cailliet, Martin, Kusher, Wolf, and Welden (1983), yet the main problem that remained was determining the time of growth band formation.

Others, such as Bass et al. (1975), Cailliet, Martin, Harvey, Kusher, and Welden (1983), Cailliet, Martin, Kusher, Wolf, and Welden (1983), and Casey et al. (1983), have tried to determine a shark's age by calculating or estimating maximum size by employing growth model procedures of Walford (1946), Beverton and Holt (1957), or von Bertalanffy (1957). Subsequent ages were then determined after applying known length data to these models. Holden (1974) suggested that the von Bertalanffy growth curve could be constructed on the basis of embryonic growth rate data and thereby estimate age at maturity for several species of dogfish, (*Mustelus* spp.). Holden (1977), Tanaka and Mizue (1979), and Francis (1981) carried this procedure further in their studies of *Mustelus* spp.

Several stains have been used by others to enhance ring patterns in elasmobranch vertebrae (Gruber and Stout 1983). Haskell (1949), Stevens (1975), and Johnson (1979) proposed silver nitrate or crystal violet staining methods as a means to enhance ring definition. De Crosta (1981), Thorson and Lacy (1982), Cailliet, Martin, Harvey, Kusher, and Welden (1983), Cailliet, Martin, Kusher, Wolf, and Weldon (1983), and Pratt and Casey (1983), have also applied the silver nitrate stain technique to a variety of sharks from California, Hawaii, Nicaragua, and the northeast United States.

DEFINITIONS

The following definitions of terms apply throughout this paper. For more detailed definitions see the Glossary.

Ring: A mark or zone on the vertebrae which may be (but not necessarily) formed once each year (analogous to annulus, see Glossary).

Marginal increment: That distance or growth from the last ring to the outermost edge of the vertebra.

Vertebral radius: That distance from the focus to the outer margin of the vertebra.

SCALLOPED HAMMERHEAD AND DUSKY SHARKS

Supposedly, some of the obstacles that stood in the way of determining the age of sharks were overcome following the work of LaMarca (1966) and Stevens (1975) who studied the concentric rings on the inner concave faces of shark vertebrae. Yet, questions still remained on how the vertebrae should be prepared, "How long should they be exposed to the stain, could the time of growth band formation be determined, and what modifications were necessary to the stain methods for best results?" Some of these questions were addressed by studying the age of the scalloped hammerhead and dusky sharks.

METHODS

Twelve of the 36 species of sharks known to occur in North Carolina waters (Schwartz 1979) were captured from April through November, between 1968 and 1981, in the Atlantic Ocean 1-3.5 km south of Shackleford Banks and 4-6.5 km east of Beaufort Inlet, N.C. All sharks were caught on unanchored 4.8 km longlines of 7.6 mm braided nylon which were fished in depths of 9-14 m. Drop lines of No. 2 chain, 1.8 m long, were snapped onto the mainline at either 9.1 or 13.7 m intervals, depending on desirability of the line to be fished high or low in the water column. Hooks were No. 9 tuna hooks. Orange plastic floats were attached to the mainline every 10 hooks to help suspend the line and keep it off the substrate. Two sets of 100 or 200 hooks per set were made daily, one east-west, the other north-south, to note capture with depth and tide.

Bait was whole fresh fish. Soak time of the line varied between 2 h for spring and fall sets to 1 h during June-September sets, when waters were the warmest. Live sharks were tagged and released. Sharks that had died fighting the line or were near death were measured (fork length), sexed, embryos removed from females, and vertebrae excised directly beneath the first dorsal fin.

Vertebral Preparation

Excised vertebrae were cleaned of excess muscle, cartilage, and either frozen or air dried under ordinary incandescent 60-W lamps for several days before storage. These "fresh" vertebrae were compared with long-term dried specimens in relation to their reliability and use in ageing, density of stain retention, and ring enhancement once stained. Both fresh and dried vertebrae proved equally receptive to staining and usable for ageing. Although no shark vertebrae that had been preserved in Formalin[2] or alcohol were used as part of this study, vertebrae shat had been preserved in Formalin and stored in 70% isopropyl alcohol for as long as 3 mo were acceptable for age determination, as they exhibited distinct rings upon staining. However, I do not recommend Formalin-preserved vertebral samples since Formalin acts as a decalcifying agent (Lillie 1954), which may etch the vertebrae and render the rings less distinct or poorly stained.

Freshly excised vertebrae were separated with a sharp knife by cutting the junction separating two adjacent vertebral centra. Vertebrae of small specimens were readily separated by simply bending the vertebral column until the juncture broke apart. Dried vertebrae were often more difficult to separate, especially from extremely large sharks, and usually necessitated careful cutting between the disks with a saw until bending or rupture separation occurred.

Vertebral fascia was removed by soaking the vertebrae in 5.25% sodium hypochlorite for approximately 1 h (Johnson 1979). Soak duration depended on size of vertebrae and how much fascia material was removed before soaking. Other methods for removing vertebral fascia connective tissue were: Soaking the vertebrae for 24 h in 0.2% sodium hydroxide and then carefully removing the connective tissue with forceps (LaMarca 1966), or soaking the vertebrae in 10 ml of 0.7% pepsin in 0.2% HCl with incubation at 39.4°C for 24 h. Soaking the vertebrae in sodium hypochlorite was adopted as the easiest method, as it saved time and was the cheapest way to prepare the vertebrae before staining.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Once cleaned, several staining methods, such as alizarin red S and anise oil, were also tried. However, only two were considered of value in enhancing growth rings: Staining with silver nitrate (Stevens 1975) and crystal violet (Johnson 1979). In general, the crystal violet method was used only when doubt, as revealed by the silver nitrate stain, existed in distinguishing growth rings. Modifications of stain time or procedure, in relation to shark fork length, for the silver nitrate and crystal violet methods are tabulated in Tables 1 and 2. Each stained vertebra was examined by two observers and when agreement as to number of rings or marginal increment distance did not correspond, the vertebra was not used in further age determination.

Table 1.—Silver nitrate and crystal violet staining procedures for vertebrae of 12 species of sharks.

Blacknose shark, *Carcharhinus acronotus*
Blacknose shark vertebrae were prepared as noted in the text. However, vertebrae should be left in the silver nitrate stain for approximately 0.5 min longer than the standard indicated time. While staining, regardless of shark size, the vertebrae should be checked every 30 s for the desired intensity of stain. Immersion staining time, depending on vertebra size, in crystal violet, which worked well, is noted in Table 2.

Blacktip shark, *Carcharhinus limbatus*
Blacktip shark vertebrae, as in the blacknose shark, had to be stained approximately 0.5 min longer in silver nitrate for best definition. The crystal violet procedure seemed to work better than the silver nitrate method for this species.

Bull shark, *Carcharhinus leucas*
Increase stain time in silver nitrate by 0.5-1 min for vertebrae of sharks larger than 2,000 mm FL. Crystal violet stain time follows that stated in Table 2.

Dusky shark, *Carcharhinus obscurus*
Of all the sharks tested, growth rings on dusky shark vertebrae were hardest to stain. Fresh vertebrae worked best, while dried vertebrae had to be immersed in the silver nitrate approximately 1-1.5 min longer than usual to pick up the stain. See Table 2 for immersion time depending on shark size.

Lemon shark, *Negaprion brevirostris*
Increase stain time in silver nitrate by 1 min for sharks larger than 2,000 mm FL as extremely large vertebrae stain slowly. Follow crystal violet stain intervals noted in Table 2.

Sand tiger shark, *Odontaspis* (*Eugomphodus*) *taurus*
Increase stain time in silver nitrate 0.5 min longer than in Table 2. Follow crystal violet stain intervals in Table 2 for large lemon sharks.

Sharpnose shark, *Rhizoprionodon terraenovae*
A change from the standard procedure is necessary because of the structure of the sharpnose vertebrae. The concave face of the sharpnose shark vertebra is deep and possesses a small hole in the middle which permits the stain to run through instead of being retained on the face. The entire vertebra must therefore be completely immersed in the silver nitrate or crystal violet stains. Failure to retain the stain on the concave face of the vertebra may jeopardize staining the first growth band.

No changes were necessary in either the silver nitrate or crystal violet methods noted in the text for the following:
Great hammerhead, *Sphyrna mokarran*
Sandbar shark, *Carcharhinus plumbeus*
Scalloped hammerhead, *Sphyrna lewini*
Silky shark, *Carcharhinus falciformis*
Spinner shark, *Carcharhinus brevipinna*

Table 2.—Suggested duration (minutes) of immersion of shark vertebrae in silver nitrate or crystal violet stain, according to fork length of shark.

Silver nitrate		Crystal violet	
Time (min)	Fork length (mm)	Time (min)	Fork length (mm)
1	-600	10	-700
1.25	700-900	12	700-1,000
1.50	900-1,000	15	1,000-1,500+
2	1,000-1,200		
3	1,500-2,590+		

Silver Nitrate Stain Method

The silver nitrate method can be used for vertebral faces that have been thoroughly cleaned and repeatedly washed for at least 5 min in distilled water after cleaning. This can be achieved by using a series of five jars with a 1 min transfer rinse in each. The 1% stain should be stored in a dark bottle and away from light when not used to prevent deterioration. Contrary to Stevens (1975), who fully immersed the vertebrae in the stain, each vertebra was positioned with one concave face uppermost. The concave vertebral face of each species examined, except for those of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, was filled to the brim with a 1% silver nitrate solution. A 2-3 ml overfill was often necessary to insure staining the extreme edge of exceptionally large vertebrae. Filling only one concave face instead of immersing the entire vertebrae conserves staining solution and permits a tidier and just as reliable operation. Sharpnose shark vertebrae have to be completely immersed in the stain since a large hole occupies the center of the vertebra, thereby permitting the stain to run out, instead of being retained as in other shark vertebrae.

The centrum should be exposed to the stain for 1-3 min, depending on size of vertebra (Table 2), and illuminated for 2 min with a 4-W UV lamp. Overstaining can easily occur, so it is advisable to check the centrum every 30 s to note the intensity of staining. While destaining with sodium thiosulfate or Kodak Farmers reducer is possible, neither method was used. Following staining, the vertebra is rinsed in distilled water and transferred to a 5% solution of sodium thiosulfate for 2 min. Stained vertebrae can be stored dry or in 70% isopropyl alcohol (Stevens 1975) once the thiosulfate has been rinsed off with distilled water.

Crystal Violet Stain Method

The crystal violet stain method consisted of cleaning the vertebra, then soaking it in 0.01% solution of crystal violet. Johnson (1979) suggested a soak time of 0.2 to 4.0 h, depending on vertebra size for teleosts. Shorter stain intervals of 10-15 min were used in this study (Table 2), with best ring definition attained if the vertebra was first overstained and then destained in 50% isopropyl alcohol, until the desired intensity of the growth rings was achieved. Destaining requires only 1 min, at most, for best results.

Reading Vertebrae

Vertebrae were measured with the centrum lying flat on the microscope stage with a calibrated ocular micrometer in a Bausch & Lomb dissecting scope under 0.7 × magnification and with overhead illumination on a dark background. Growth rings appeared as opaque and translucent zones (see Glossary). Distances from the core to and between each visible stained ring and from the core to the outer edge of the centrum were measured with the interface of the centrum at an angle to the field of view. Growth rings were best discernible immediately following staining. Immersion in water or glycerol did not increase ring intensity appreciably.

To gain insight into when growth rings were formed, vertebrae were grouped by month of capture. Size of growth rings was measured, noting when large or small incremental variations occurred between rings, especially near the edge of the centrum.

The relationship between vertebral radius and fork length was determined for scalloped hammerheads and dusky sharks using linear regression, rather than a curvilinear relationship often used for other fishes (Rounsefell and Everhart 1953). This was expressed by the formula $y = a + bX$ where X was vertebral radius (in millimeters) and y was shark fork length. Substitution of the measurement distance from core to each growth ring, for each species, into the linear relationship formula permitted back calculations of length for each estimated age observed. All statistical inferences were made with a significance level of $\alpha = 0.05$.

In this report, I concentrate on the age and growth of the scalloped hammerhead and dusky sharks, as those species were the most abundant of the 12 species captured. Growth and back-calculation estimates for most of the other 10 sharks studied will await adequate samples of vertebrae and are outside the scope of this study.

RESULTS AND DISCUSSION

Even though criteria used to estimate age and growth were first established by studying teleost fish scales (Van Oosten 1929), the same criteria can be adapted to estimate a shark's age and growth. These criteria (Jolley 1977; Brothers 1983; Smith 1983) can be summarized as follows: 1) The ageing structure must develop early in life and remain constant in number and identity, 2) growth of the structure must be proportional to growth of the fish, 3) growth rings must be formed at approximately the same time each year, and 4) theoretical lengths or weights back calculated from various growth rings must have positive correlations with empirical data.

Criterion 1 was easily met as the vertebral column of sharks develops early in life. The relationship of proportional vertebral growth to growth of fish was noted by plotting fork length against vertebral radius. These data exhibited a linear (range for all species, $r = 0.91$-0.97, and for scalloped hammerhead and dusky sharks, Fig. 1) rather than a curvilinear relationship. Preliminary attempts to resolve Criterion 3 by examining the marginal growth ring on vertebrae suggested that hammerhead shark vertebral growth rings are formed annually, whereas

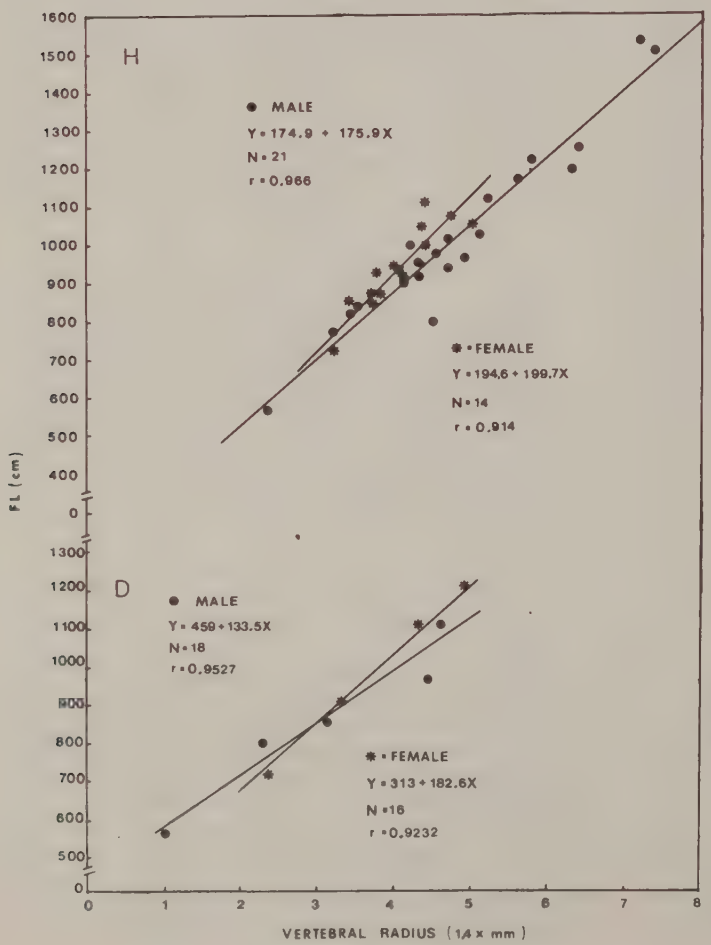

Figure 1.—Relationship between fork length (cm) and vertebral radius (mm) for male and female scalloped hammerhead (H, top) and dusky sharks (D, bottom).

those for the dusky shark may be formed one or more times a year. Casey's et al. (1983) observations of false checks and other rings in vertebrae of sandbar sharks may explain the trend observed for dusky sharks. However, more definitive results for both species awaits adequate samples of the full size range of each sex. Slight differences between back calculations of the theoretical shark length and actual fork lengths suggested a Dahl-Lee effect (discussed later).

Scalloped Hammerhead

Scalloped hammerhead sharks appear in North Carolina inshore waters, near Shackleford Banks, from May to October, and occasionally remain until November. Peak abundance occurs from July to mid-August. There was a significant linear relationship between fork length and vertebral radius for male (N = 21) and female (N = 14) hammerhead sharks (Fig. 1, r = 0.96 and 0.91 for males and females, respectively). These relationships were expressed by the linear regressions: Males y = 175.9 + 174.9 X, and females y = 124.6 + 199.7 X. Maximum age for males was estimated to be 8 yr, while females appeared to be at least 5 yr old.

Back calculations of fork length at estimated age produced relatively close agreement with observed data for the female scalloped hammerhead data (Fig. 2), while back calculations for males were usually smaller than the actual observed measurements (Fig. 2, Table 3). In such cases, small overall sample sizes may explain the disagreement noted.

While Bass et al. (1975) calculated that the maximum size for male hammerhead sharks should be 2,950 mm TL, Clarke (1971) reported a 3,090 mm TL female from Hawaii. Gilbert (1967) speculated that the maximum length for scalloped hammerhead shark was probably between 3,700 and 4,000 mm TL. Gudger (1947) cited a 4,560 cm TL (15 ft), an unlikely size, hammerhead shark from Australia. Our largest specimen was a mature male scalloped hammerhead shark of 1,560 mm FL;

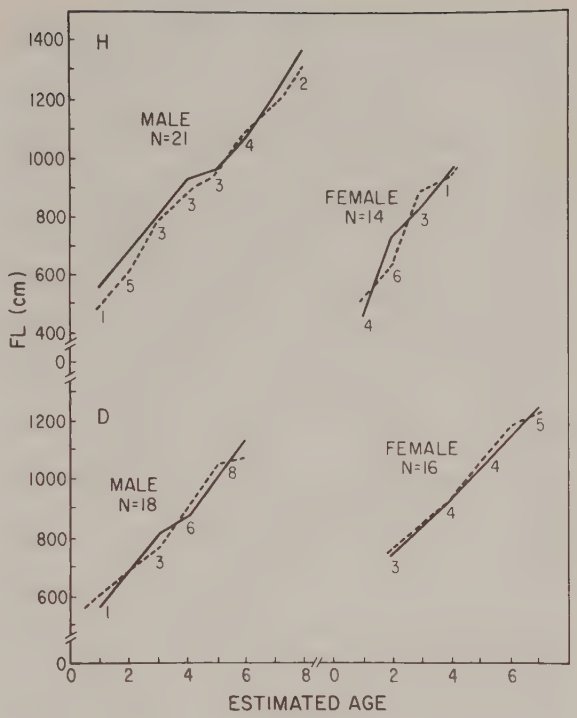

Figure 2.—Actual (solid line) and back-calculated (dashed line) fork lengths for male and female scalloped hammerhead (H, top) and dusky sharks (D, bottom). Numbers refer to sample size.

Table 3.—Back-calculated fork lengths (mm) for male and female scalloped hammerhead sharks.

Estimated age	N	Fork length at vertebral ring 1	2	3	4	5	6	7	8
		Male							
1	1	495.7							
2	5	495.5	621.8						
3	3	501.2	667.8	783.6					
4	3	499.5	631.9	762.3	894.2				
5	3	498.5	634.5	779.1	916.1	976.5			
6	4	505.3	636.5	800.9	898.2	974.6	1,073.6		
7	—	—	—	—	—	—	—	—	
8	2	508.2	621.9	814.4	910.8	997.9	1,085.4	1,172.8	1,242.8
$\bar{x}$ FL		500.4	635.1	787.2	903.8	980.4	1,077.5	1,172.8	1,242.8
		Female							
1	—	—							
2	4	466.9	636.7						
3	6	454.1	609.8	773.6					
4	3	424.2	583.9	748.7	933.4				
5	1	384.2	563.9	743.7	943.4	1,043.2			
$\bar{x}$ FL		446.4	608.7	763.1	935.9	1,043.2			

otherwise, most of the scalloped hammerhead sharks examined were immature.

Dusky Shark

Dusky sharks appear in the Shackleford Banks inshore waters in early May and remain until late October. Peak abundance occurs in May-early June and again in early September. During July and August they apparently move north or south along the Atlantic coast, as they are usually replaced by other carcharhinids. Similar north-south movements along southeastern Natal have been reported by Bass et al. (1973).

The vertebral radius to fork length relationships for 18 male and 16 female dusky sharks, like those for hammerhead sharks, also exhibited a significant linear relationship (Fig. 1, $r = 0.99$ male, 0.92 female). These relationships are expressed by the formulas: Males $y = 459.0 + 133.5\ X$, and females $y = 313.0 + 182.6\ X$. Males attained a maximum fork length of 1,120 mm by estimated age 6, while female maximum lengths were 1,215 mm at estimated age 7.

Back calculations of fork length at estimated age for dusky sharks agreed with observed data for both sexes, except that the largest male and female fork lengths were slightly underestimated by the regressions (Fig. 2, Table 4). These results suggested a possible Dahl-Lee effect among the specimens studied. Again, small sample sizes may also account for these differences.

Springer (1960) recorded 3,400 mm TL male and 3,650 mm TL female dusky sharks in the western Atlantic, while those in the Indian Ocean attained total lengths of 3,240 mm for males and 2,570 mm for females (Bass et al. 1973).

Too few vertebrae were available for 10 other species of sharks to adequately estimate age by the silver nitrate method or to permit regression analyses or back calculations at this time. Also, little can be reliably said regarding month of growth ring formation for those 10 species. Estimated ages from the largest sized vertebra and maximum fork length (mm), by species, were: Blacknose shark, *C. acronotus*, 7 yr (male maximum 1,340 mm, female 1,195 mm); spinner shark, *C. brevipinna*, 7 yr (male 1,640 mm, female 1,571 mm); silky shark, *C. falciformis*, 5 yr (male 1,052 mm, female 1,055 mm); bull shark, *C. leucas*, 10 yr (male 2,460 mm); sandbar shark, *C. plumbeus*, 5 yr (male 1,000 mm, female 1,130 mm); lemon shark, *Negaprion brevirostris*, 14+ yr (female 2,421 mm); sand tiger, *Odontaspis taurus*, 8+ yr (male 2,161 mm); Atlantic sharpnose, *Rhizoprionodon terraenovae*, 6 yr (male 890 mm, female 895 mm); and great hammerhead, *Sphyrna mokarran*, 14+ yr (male 3,660 mm). The male sandbar shark age-growth data agree well with that noted by Casey et al. (1983), whereas their females were estimated to be age 7 at 1,100 mm FL. Gruber and Stout (1983) noted that von Bertalanffy growth estimates of a 310 cm TL lemon shark would be at least 9 yr old. While they gave no formula for converting total length to fork length, a specimen of about 290 cm FL would either be a faster growing shark, or the von Bertalanffy growth model overestimates growth. Gruber and Stout (1983) believed the latter is true. Thorson and Lacy's (1982) largest male bull shark (201 cm TL) exhibited 10 vertebral rings. Bull sharks examined in this study were near the maximum total length reported by Schwartz (1959, 1960), yet would be 5 or more years younger than those determined by Thorson and Lacy. These discrepancies suggest more work is needed.

Table 4.—Back-calculated fork lengths (mm) for male and female dusky sharks.

Estimated age	N	Fork length at vertebral ring						
		1	2	3	4	5	6	7
Male								
1	1	592.5						
2	—	—	—					
3	3	619.2	712.7	819.5				
4	6	504.9	712.7	766.1	872.9			
5	—	—	—	—	—	—		
6	8	632.6	712.7	766.1	952.9	1,006.4	1,073.1	
$\bar{x}$ FL		585.5	712.7	775.5	918.6	1,006.4	1,073.1	
Female								
1	—	—						
2	3	586.9	732.9					
3	—	—	—	—				
4	4	568.6	696.5	787.8	915.6			
5	—	—	—	—	—	—		
6	4	550.4	659.9	806.0	998.6	1,061.7	1,152.9	
7	5	568.6	659.9	824.3	952.1	1,061.7	1,134.7	1,207.7
$\bar{x}$ FL		567.5	682.7	807.4	954.4	1,061.7	1,142.9	1,207.7

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SUMMARY PAPER

Age and Growth of the Shortfin Mako, *Isurus oxyrinchus*[1]

HAROLD L. PRATT, JR. and JOHN G. CASEY[2]

There have been few attempts to age the larger pelagic elasmobranchs (Aasen 1963; Stevens 1975). Efforts to verify ageing estimates using more than one method are also uncommon for sharks. Whole centra have been used by Daiber (1960), Taylor and Holden (1964), and Stevens (1975) to estimate the age of sharks. Elasmobranchs have also been aged from tag-recapture data (Holden 1972; Thorson and Lacy 1982) and length-frequency analysis (Olsen 1954; Aasen 1963). The shortfin mako, *Isurus oxyrinchus*, is an important member of the pelagic community in temperate waters of the world's oceans, but many details of its life history, including age and growth, remain undescribed.

Shortfin makos were obtained from hook and line catches of sharks at sportfishing tournaments from New Jersey to Rhode Island and from cruises aboard research and commercial longlining vessels (1965-81). Age and growth rate of shortfin mako captured between 1961 and 1981 in the western North Atlantic was determined using four methods: 1) Temporal analysis of length-month information, 2) results of tagging data, 3) length-frequency analysis, and 4) ring counts on vertebrae. All results are reported in fork length[3] (FL, tip of snout to fork of tail). Length-month data were transformed into a growth curve using a technique similar to one used by Mackintosh and Wheeler (1929, Fig. 1). A total of 175 juvenile shortfin makos < 175 cm FL were used to determine size, time of birth, and early growth rate for young shortfin makos. Data on growth from 40 tag-recaptured shortfin makos were obtained from over 800 shortfin makos that were tagged by us and cooperative fishermen in the western North Atlantic, principally between Cape Hatteras and Cape Cod (1961-81). To quantify growth, tag-recapture information was tabulated in 20 cm FL groups based on the size at tagging. Growth per month and growth per year were calculated. Length and weight frequency curves were compared and lengths were chosen for analysis as they better revealed frequency modes. With sufficient grouping of years, shortfin mako length-frequency distributions display adequate polymodality for mode dissection using a Du Pont[4] 310 curve resolver (Müller 1966). Counts of growth rings on vertebral centra stained with silver nitrate were employed to back-calculate lengths at estimated ages for both sexes separately and for the full size range (69-328 cm FL).

Results of the length-month information, relating size to estimated age for 175 juveniles < 175 cm FL, were used to determine size at birth (60-70 cm FL), time of birth (late spring), and early growth rate (50 cm/yr for ages 0-1, 32 cm/yr for ages 1-2). This growth rate was used as a basis for interpreting the results of other methods. Annual growth rates were also calculated from 40 tag-recaptured shortfin mako sharks. These compared well with length-month analyses. Length-frequency modes extended age estimates to intermediate-sized shortfin makos (Fig. 2). Interpretation of back-calculated ages was based on the hypothesis (assumption) that two rings are formed on the centrum each year. Age estimates based on two rings per

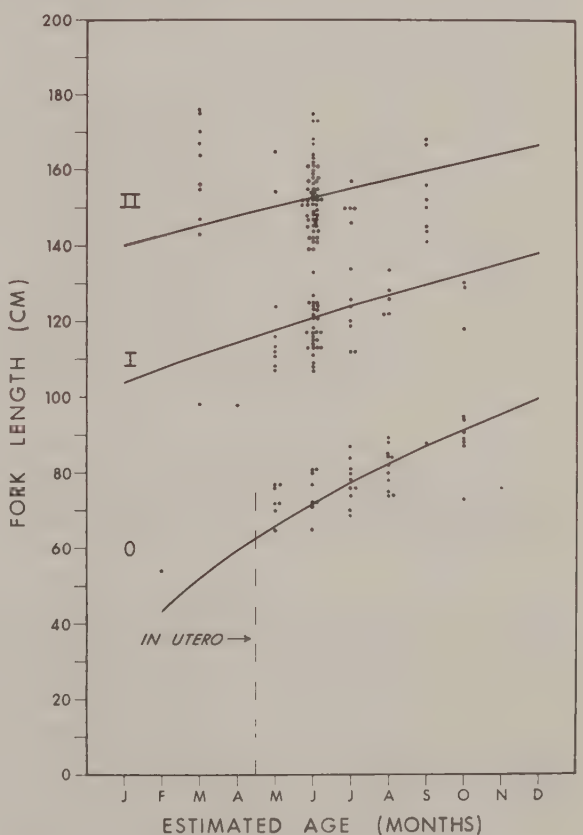

Figure 1.—Length-month diagram of juvenile shortfin makos (age 0-2) with fitted growth curve based on cluster analysis.

[1]This is MARMAP Contribution MED/NEFC 82-3 and was summarized from a manuscript published in the Canadian Journal of Fisheries and Aquatic Sciences, Vol. 40, 1944-1957.

[2]Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA, Narragansett, RI 02882.

[3]Fork length = −1.931 + 0.929 (total length), r^2 = 0.99, SD = 2.57.

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

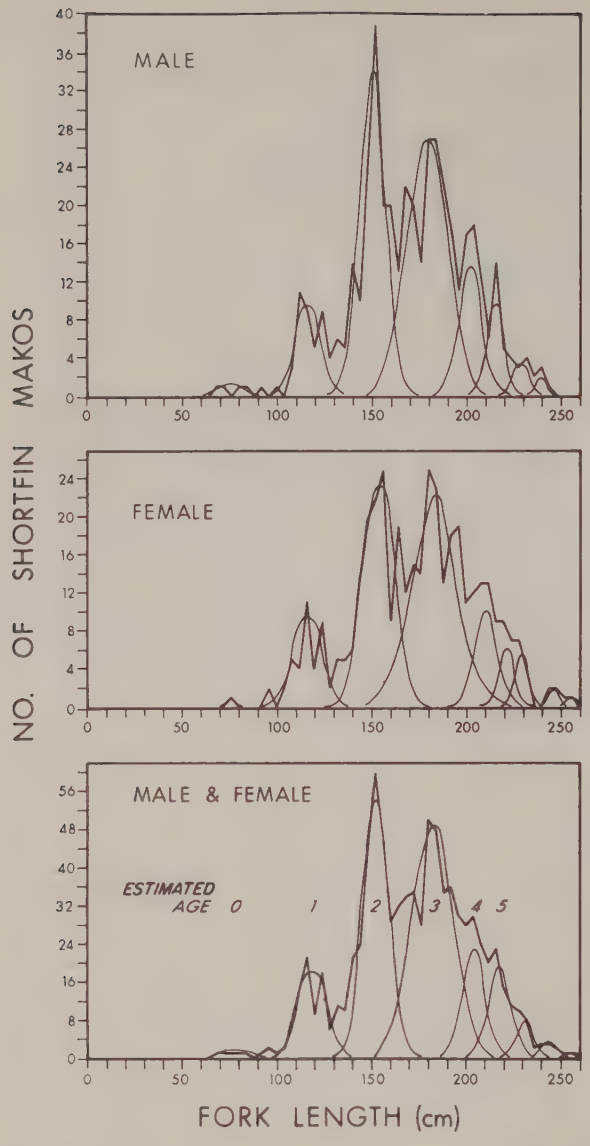

Figure 2.—Length-frequency polygons of fork length vs. number of shortfin makos caught each June from 1961 to 1981 with normal (Gaussian) curves overlaid to represent year class modes. Males, n = 448; females, n = 395; males plus females, n = 848; i = 4.

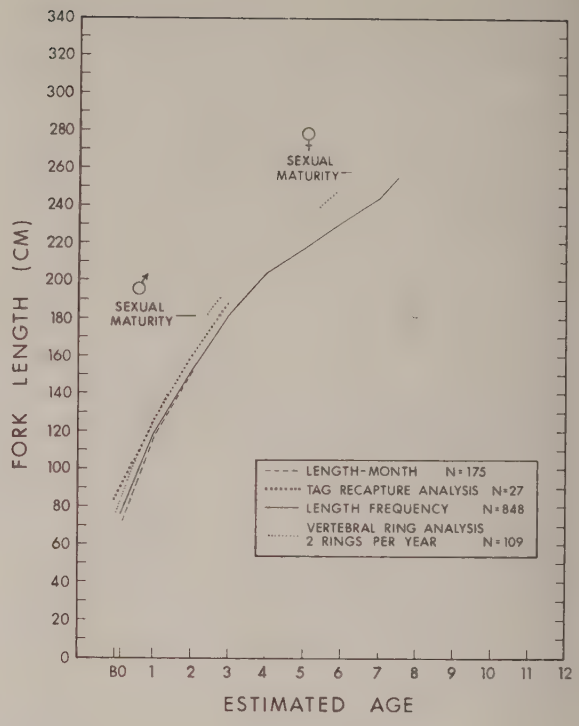

Figure 3.—Composite growth estimates from four methods for shortfin makos from the northwest Atlantic (1961-81).

year (Table 1) agreed well with results from other methods (Fig. 3).

Males and females were found to have a similar growth rate even though females grow much larger than males. The oldest female in the sample was 11.5 yr at 328 cm FL. The oldest male was 4.5 yr at 225 cm FL. The von Bertalanffy asymptotic growth function adequately described shortfin mako growth: Female L_∞ = 345 cm FL, k = 0.203, t_0 = −1 yr; male L_∞ = 302 cm FL, k = 0.266, t_0 = −1 yr.

Our approach to shortfin mako age and growth utilized two methods that provided a basis for verifying the interpretation of the two more traditional methods. The comparison and integration of these methods is essential in achieving an accurate understanding of shortfin mako age and growth. The growth curve derived from length-month analysis relates growth to time for juveniles, and with tagging results confirms the year

Table 1.—Back calculations of fork length (cm) at estimated ages based on vertebral measurements for male, female, and combined sexes of shortfin makos from the northwest Atlantic Ocean. B = birth. Combined sexes include six shortfin makos of unknown sex.

												Ring number													
	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sex												Estimated age (yr)													
(n)	B	0		1		2		3		4		5		6		7		8		9		10		11	
Females																									
(49)	70	76	99	122	145	167	185	203	210	220	232														
Males																									
(54)	76	84	104	127	149	169	185	196	207	217	226	231	241	249	258	267	274	276	295	306	311	316	320	335	301
Males and females																									
(109)	74	81	102	125	148	168	184	197	206	216	226	231	241	250	260	269	276	278	297	309	314	318	323	338	303

assignments of the less empirical length-frequency and vertebral ring analyses. Growth data derived from tag-recapture information, which is also limited to smaller sizes, provides a growth rate very similar to those determined using the length-month analysis. Resultant growth estimates from length-month, tag-recapture, length-frequency, and vertebral-ring analyses were not significantly different ($\alpha = 0.05$) when compared with a test of homogeneity of slopes. Growth rates from earlier analyses are used only to introduce biological structure into the more subjective length-frequency and vertebral-ring analyses. Vertebral-ring age determinations were used to calculate the von Bertalanffy growth function since it covered the greatest range of length data, including mature females, and is in agreement with the other analyses.

Growth of the shortfin mako is rapid when compared with most other sharks. It grows at nearly twice the rate of the porbeagle, *Lamna nasus* (Aasen 1963), and much faster than typical carcharhinid sharks (Thorson and Lacy 1982; Casey et al. 1983; Gruber and Stout 1983). Its growth is more commensurate with that of other species of pelagic fish such as dolphin, *Coryphaena hippurus* (Beardsley 1967), blue sharks, *Prionace glauca* (Stevens 1975), tunas and billfishes. Cailliet et al. (1983), using 44 shortfin makos and one ageing technique, vertebral ring enhancement, estimated a slower growth rate for the Pacific shortfin mako. If two vertebral rings were assumed to form each year in Pacific shortfin makos, as well as Atlantic shortfin makos, then Cailliet's growth estimations would approach ours.

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Preliminary Studies on the Age and Growth of Blue, *Prionace glauca*, Common Thresher, *Alopias vulpinus*, and Shortfin Mako, *Isurus oxyrinchus*, Sharks from California Waters

GREGOR M. CAILLIET,[1] LINDA K. MARTIN,[1] JAMES T. HARVEY,[2]
DAVID KUSHER,[1] and BRUCE A. WELDEN[1]

ABSTRACT

Two methods of enhancing growth bands on vertebral centra—silver nitrate impregnation and X-radiography—have proven to be successful when used on centra from 130 blue, *Prionace glauca*, 143 common thresher, *Alopias vulpinus*, and 44 shortfin mako, *Isurus oxyrinchus*, sharks. Bands were counted and measured, and these data were used to construct growth curves based on the von Bertalanffy and logistic growth models. The problems of verification of these counts, and validation of the periodicity of band formation, have been identified and are discussed in relation to the growth curves generated for each of these three species. Our results and other available information indicate that these elasmobranchs grow relatively slowly, reaching their asymptotic lengths at 20 yr of age for blue sharks, and between 45 and 50 yr for shortfin mako and common thresher sharks. They have a large size but relatively early age of first reproductive maturity, and low fecundities. This combination of traits could make them susceptible to overfishing.

INTRODUCTION

Commercial fishing for elasmobranchs is increasing rapidly in California. For example, the United States Department of Commerce (1978-80) reported that blue shark, *Prionace glauca*, landings in San Pedro, Calif., have increased from virtually nothing in 1978 to over 188,000 lb in 1980. Similar trends have occurred for common thresher, *Alopias vulpinus*, and shortfin mako, *Isurus oxyrinchus*, sharks. Landings of common thresher and shortfin mako increased from 15,500 lb and 1,129 lb in 1978 to 994,000 lb and 62,000 lb, respectively, in 1980. Thus, commercial fishing of these three species has increased over the past few years, and they now comprise over 87% of the total shark landings in San Pedro. Historically, sharks were used primarily for their oils, for reduction (Byers 1940), and for the vitamins in their livers (Frey 1971). Today, however, their principal use is for food.

A major problem that arises with this increased commercial use of elasmobranchs is the lack of life history information necessary to ensure effective management. For example, age determination has not been evaluated sufficiently for the majority of elasmobranchs in California, and, therefore, such critical information as age at first maturity is not known.

The usual means of age determination in bony fishes, by examining scales, otoliths, or bones, are not applicable to elasmobranchs. However, the evidence that does exist indicates that growth rates in sharks may be slow, compared with many teleosts, with size at first sexual maturity estimated at approximately 60 to 90% of the asymptotic length (Holden 1977). Because of these growth and reproductive traits, intensive fisheries directed toward subadults could deleteriously affect the total population size of sharks very quickly, assuming a close relation between stock and recruitment (Holden 1973, 1974, 1977). Without information on size and age at which reproduction first occurs, effective management measures, such as setting size restrictions, would be difficult to implement.

Recently, several techniques have been used to estimate ages of elasmobranchs by counting bands laid down concentrically in their vertebral centra (Stevens 1975; Cailliet et al. 1983). However, nothing has been done to estimate age or growth of blue, common thresher, and shortfin mako sharks in California waters. Therefore, our objectives in this study were to use recently developed techniques to enhance growth bands on centra for these three west coast pelagic sharks, make estimates of age based on counts from these structures, and construct preliminary growth curves. Finally, because these pelagic sharks appear to range widely over the oceans (Strasburg 1958), very little information has been gathered that could validate the presumed annual nature of bands in their vertebral centra. We attempted to use what little information was available on their size and reproduction to evaluate our growth curves.

MATERIALS AND METHODS

Most blue sharks were collected between September 1974 and October 1977 in Monterey Bay, Calif., by longline and hook and line using 2 m stainless steel leaders baited with either anchovy or squid. Most collections of common thresher and shortfin mako sharks and several specimens of blue sharks were obtained from commercial fisheries in southern California and from the California Department of Fish and Game pelagic gill net observer program. Additional preserved specimens of all three species were obtained from several California museums.

[1]Moss Landing Marine Laboratories, Moss Landing, CA 95039.
[2]Moss Landing Marine Laboratories, Moss Landing, Calif.; present address: Oregon State University, Marine Science Center, Newport, OR 97365.

All sharks were measured and weighed and their sex and reproductive status noted, if possible. The main measurements used were total length (TL), fork length (FL), and alternate length (AL, the distance between the origins of both dorsal fins). All length measurements were converted to total length for uniformity using conversion factors based upon measurements from the literature (Bigelow and Schroeder 1948; Applegate 1977) and from our own specimens. For age determination, a section of the vertebral column was removed, usually just anterior to the first dorsal fin, because this appears to be the area where vertebrae are largest and most calcified (Ridewood 1921). However, in some cases, such as common threshers collected from fish markets, we could only obtain caudal vertebrae from carcasses. Each section, usually consisting of 8-12 vertebrae, was frozen in a plastic bag or was stored as long as several months in 50% isopropyl alcohol until it was analyzed.

For all three species, a piece of the defrosted vertebral column was cleaned using a combination of steps. First, the haemal arch and lateral processes were removed, and most of the connective tissue was picked off with forceps to expose the surface of the centra. Then, several centra were soaked for approximately 5 min in distilled water, followed by air drying or by soaking in bleach to further facilitate the removal of connective tissue from the centrum. For larger centra, a longer soaking time was needed, and immersion intervals ranged from 5 to 30 min. The centra of the blue sharks were then soaked in a concentrated solution of formic acid for 2 to 4 min to remove any remaining traces of bleach and to etch the centrum surface. For the centra of common thresher and shortfin mako sharks that were X-rayed, the formic acid treatment was not necessary. Centrum diameters (millimeters) were measured for each specimen of common thresher and shortfin mako, and the relationship between centrum diameter and total length of the fish was determined with regression analysis. This has already been done for blue sharks by Stevens (1975). All statistical inferences were made with a significance level of $\alpha = 0.05$.

The ageing technique used for blue sharks was modified from a procedure attributed to Von Kossa (Stevens 1975). This basically involved replacing the calcium salts in the centrum with silver, providing distinct silver-impregnated bands, which become quite dark after illumination under ultraviolet light. After cleaning, these centra were rinsed in distilled water for approximately 15 min, then immersed in a 1% silver nitrate solution, and immediately placed in a chamber where they were illuminated by an ultraviolet light source. The length of light exposure ranged from 3 to 15 min, depending upon centrum size. The centrum was then rinsed again in distilled water to remove excess silver nitrate. A dissecting microscope, with illumination focused laterally on the centrum, was used to count bands. Because staining clarity can be inconsistent, several centra from each specimen were stained and counted by two or three different readers for replicate analysis. Once a concensus was reached regarding these counts on the newly stained centra, they were soaked in a 5% sodium thiosulfate solution for 2 to 3 min. This procedure removed excess silver and fixed the chemical substitution. Because fixation also eradicated the very narrow rings, counts were made before and after fixation to estimate this bias. The final step was storage in 70% iospropyl alcohol. Also, the radius, defined as the distance from the center of the focus to the outer edge of each light band, was measured so they could be compared with similar measurements made by Stevens (1975).

The cleaned centra from the common thresher and shortfin mako sharks were X-rayed using a Hewlett-Packard[3] Faxitron Series X-ray system (Model No. 43805N) with Kodak Industrex M film (Readypack M-2). These X-radiographs were viewed through a dissecting microscope using transmitted light from below.

For both of these techniques, procedures for counting the concentric lines were standardized. We defined any concentric line found on a centrum as a "ring." We further defined "band" as a group of rings (Cailliet et al. 1983). Two kinds of bands occurred: Those that were transparent (translucent, see Glossary) with transmitted light and those that were more opaque. In silver nitrate impregnated centra, opaque bands appeared black, and in X-rays they were white. We assumed that these bands were more heavily mineralized and represented summer growth on the centrum (Jones and Geen 1977). To insure the accuracy of band counts, at least two observers made independent counts of the opaque bands on each centrum. If these initial counts did not agree and additional readings did not result in a concensus, the centrum was not used for age analysis.

For simplicity and the widest applicability of this preliminary age information, we fit our data on age and length for all three species to the von Bertalanffy (1938) growth equation using methods for calculating the parameters L_∞, k, and t_0 from Allen (1966), Gulland (1969), and Everhart et al. (1975). Those parameters producing the best fit (least mean square error) from one of these methods were then selected to plot the growth curve for each species. These parameters were calculated for all individuals of each species combined and separately for male and female blue and common thresher sharks. Sexes were not separated for shortfin mako sharks, because the data set consisted of only 44 fish. Growth was characterized for all three species by plotting individual total lengths (TL) against estimated ages, and by plotting the predicted von Bertalanffy growth curve based upon the parameters L_∞, k, and t_0 for combined sexes. For the shortfin mako, we also used the logistic growth equation (Ricker 1979).

As an initial approximation of the temporal periodicity of band formation, we plotted size-frequency histograms of all specimens of each species collected during the entire study period and plotted above these the means and standard deviations of total length at estimated ages based on band counts. Visually, we then compared mean size at estimated age with the corresponding modes in the size-frequency distribution.

For the blue shark, we compared our growth curve with information presented for North Atlantic blue sharks by Stevens (1975, 1976), and we sent two of our centra to him for independent band counts. Our shortfin mako shark growth data were compared with those presented by Pratt and Casey (1983) for the same species in the western Atlantic Ocean. For all three species, we also compared the size and age at birth, first maturity, and the maximum size reported in the literature with those values estimated from our growth curves to gain insight into the accuracy of our counting methods.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

RESULTS AND DISCUSSION

Blue Shark

We caught a total of 120 blue sharks between 1974 and 1977, with an additional 42 specimens coming from museum collections and the commercial catch in southern California taken over a wider range of years. The Monterey Bay collections produced specimens ranging from 958 to 2,045 mm TL, and fish smaller and larger than these sizes were added from the additional sources. The resulting size range collected was between 300 and 2,705 mm TL (Fig. 1). Because blue sharks are born at approximately 400 mm TL and reach a reported maximum size of about 3,962 mm TL (Bigelow and Schroeder 1948; Tucker and Newnham 1957; Strasburg 1958; Miller and Lea 1972; Hart 1973; Pratt 1979), our sample sizes are low for the smallest and largest size classes. Although the blue shark is known to make extensive, sexually segregated migrations (Strasburg 1958; Beckett 1970; Stevens 1976), our samples suggest that the larger individuals are uncommon off central California, or are not as vulnerable to commercial gear. Even with extensive collecting efforts, blue sharks over 2,600 mm TL are quite rare in northeast Pacific waters (Strasburg 1958).

Both silver nitrate and X-radiography produced clear bands (Urist 1961; Cailliet et al. 1983), but the silver nitrate technique was chosen to age blue sharks (Fig. 2), because it was the first technique available and it worked consistently well; it was also used by Stevens (1975) on this species. Because we counted bands in centra and not the finer rings, all counts taken before fixing in sodium thiosulfate were identical to those taken immediately after.

The von Bertalanffy growth curve for the 130 blue sharks we aged, which ranged between 280 and 2,521 mm TL, rose steeply and leveled at an estimated TL of 2,655 mm for both sexes combined (Fig. 3). Males were estimated to reach a larger asymptotic size (2,953 mm TL) than females (2,419 mm TL), but as in Stevens' (1975) study, there were insufficient samples to recognize significant differences in male and female growth rates. The oldest fish in our sample was a 2,450 mm TL male that had nine bands, while the youngest were two near-term embryos that had no bands and were between 350 and 400 mm TL.

The male asymptotic length was close to that of the largest specimens commonly collected in the Pacific (around 3,100 mm TL; Strasburg 1958), but was considerably smaller than the largest reported blue shark (3,962 mm TL; Bigelow and Schroeder 1948). Extrapolating from our von Bertalanffy growth curve for combined sexes, a fish at the asymptotic length of 2,655 mm TL would be approximately 20 yr old. With additional larger specimens, our estimate of asymptotic length might increase, and this would agree more with the maximum reported size, unless Pacific blue sharks do not grow comparably with those in the Atlantic. Until larger specimens are obtained, the maximum age attained by the blue shark will remain unknown.

Our estimate of size at birth (435 mm TL), derived from the von Bertalanffy growth curve, was between the reported sizes of free-living young (340 and 530 mm TL; Bigelow and Schroeder 1948; Tucker and Newnham 1957; Strasburg 1958; Hart 1973; Pratt 1979). Also, the mean sizes of the younger age classes corresponded to the size modes of blue sharks collected (Fig. 1). With larger and older fish, this relationship deteriorated, perhaps due to our small sample size or mixing of several age classes into a larger size class due to different individual growth rates and slower growth rates in general.

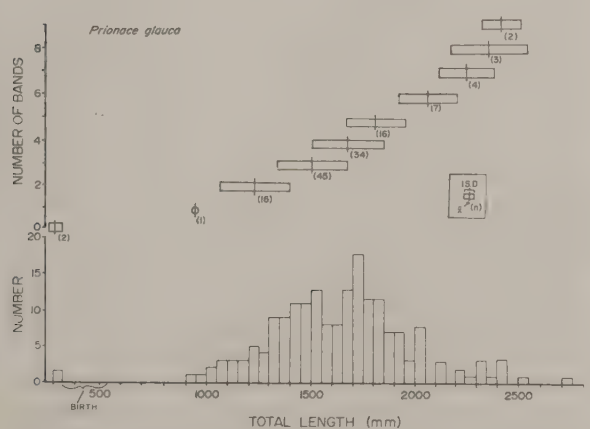

Figure 1.—Size-frequency histogram of blue sharks collected from California waters used for age determination, with the means (vertical lines) and standard deviations (horizontal bars) of the lengths of all fish placed in a single age category shown above the pertinent size-frequency axis. Sample sizes are in parentheses.

Figure 2.—Centra from blue sharks treated with silver nitrate. (a) From a small (972 mm TL) free-living male, centrum diameter 7 mm, estimated age 0 + ; and (b) from an adult male (2,401 mm TL), centrum diameter 24 mm, estimated age 7.

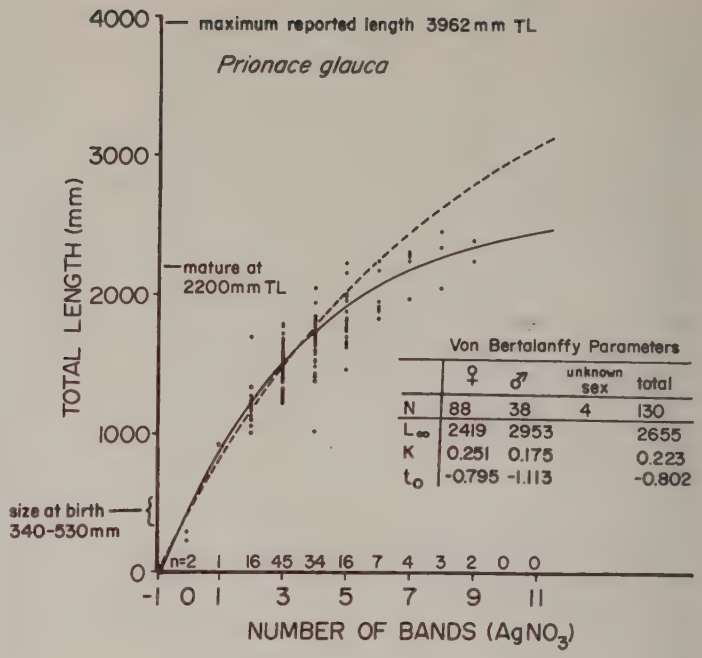

Figure 3.—Von Bertalanffy growth curve for 130 blue sharks collected in California waters where age was estimated using silver nitrate. Dots represent individuals of both sexes, and von Bertalanffy parameters for males, females, and the total sample are given in the insert. Dashed growth curve is based on Stevens (1975) and references used for size at birth, size at maturity, and maximum size are given in text.

Stevens (1975), using size frequencies and the silver nitrate technique on centra of 81 blue sharks of both sexes from the eastern North Atlantic, produced a von Bertalanffy growth curve that corresponds to ours for the first three or four age classes, but his estimates of mean length of sharks between estimated ages 5 and 6 were higher. Stevens (1976), from tag-recapture size information, estimated growth at approximately 320 mm/yr for sharks between 800 and 2,040 mm TL, which is higher than our average estimate of about 210 mm/yr taken from the growth curve for similarly sized blue sharks. Also, our measurements of radii in centra were somewhat smaller at higher band counts than those of Stevens (1975), providing further evidence that the growth rates of blue sharks off California may be a bit less than those found in the eastern North Atlantic. Stevens (1975) used both his centrum band counts and Aasen's (1966) size-frequency data to generate growth curves and to estimate asymptotic lengths for both sexes combined of 3,950 and 4,230 mm TL, respectively, which are both considerably higher than the asymptotic length we derived from observed sizes and ages (2,655 mm TL for both sexes combined; Fig 3.). The counts of bands on two centra sent to Stevens were identical to those made by us. In addition, his estimate of yearly growth rates from recaptured blue sharks (Stevens 1976) corresponds with our growth curve up to about 2,000 mm TL, and his (1975) size and age data fit within the range of observations we have found for similar age classes. Therefore, we feel that the differences between these two studies could partly be due to the methods used to calculate the von Bertalanffy growth parameters. For blue sharks, we followed the methodology of Allen (1966), while Stevens (1975) used the Ford-Walford plot to calculate asymptotic length. Of course, blue sharks living under different oceanic conditions could exhibit different growth characteristics.

According to Pratt (1979), the blue shark reaches maturity at approximately 2,200 mm TL, which, according to our age estimates, is 6 or 7 yr of age. Thus, blue sharks become reproductively mature at about 56% of their maximum reported size and 83% of our estimated asymptotic length. This conforms to Holden's (1977) generalization that most elasmobranchs become mature at about 60 to 90% of their asymptotic lengths. However, using the estimated age of 20 yr at asymptotic length, blue sharks first become sexually mature at an age that is only 30 to 35% of their projected life span.

Common Thresher Shark

A total of 167 common thresher sharks was collected from the southern California gill net fishery and museum collections. The specimens ranged in size from embryos of 360 mm TL and free-living juveniles of about 1,450 mm TL to adults up to 5,733 mm TL (Fig. 4). Because common threshers are reported to reach maximum lengths of 6,096 mm TL (Bigelow and Schroeder 1948) to 7,600 mm TL (Hart 1973), our sample does not contain sufficient representatives of the larger size classes. However, Hart (1973) reported that 13- to 16-ft (3,800-4,900 mm TL) specimens are "common" in the northeastern Pacific, and, therefore, we have some representatives of the locally occurring larger size classes of this species. As with blue sharks, the common thresher shark is thought to make large-scale migrations, and their distribution patterns will influence the sizes available at any one location (Strasburg 1958).

Although both techniques produced clear bands, the X-radiography technique was chosen to age common thresher sharks, because it worked consistently well and many vertebrae could be easily processed in a short time (Cailliet et al. 1983). Bands were most easily seen in X-radiographs of centra from small common thresher sharks; however, with larger sharks, the banding patterns at the outer edge of the centra were slightly more difficult to identify and count (Fig. 5). A significant ($r^2 = 0.90$; $P < 0.01$) linear relationship was found between total length and diameter of caudal centra in common thresher

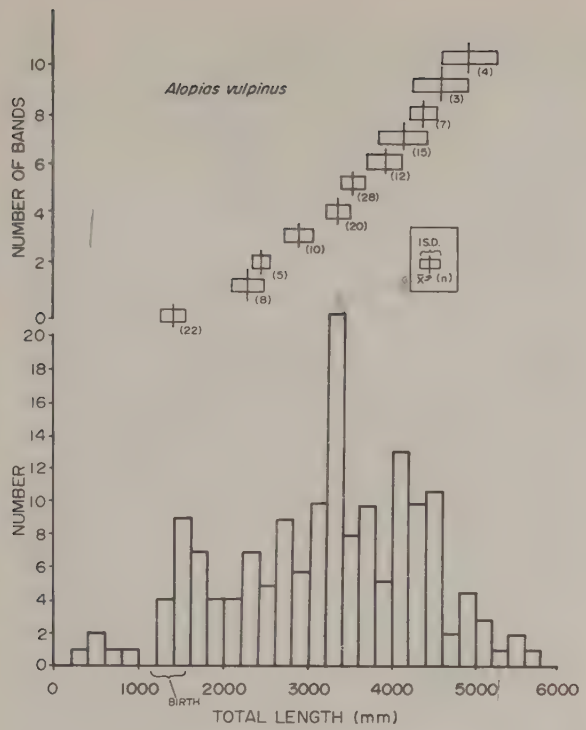

Figure 4.—Size-frequency histogram of common thresher sharks collected from California waters and used for age determination. Means (vertical lines) and standard deviations (horizontal bars) of the lengths of all fish placed in a single age category are shown above the pertinent size-frequency axis. Sample sizes are in parentheses.

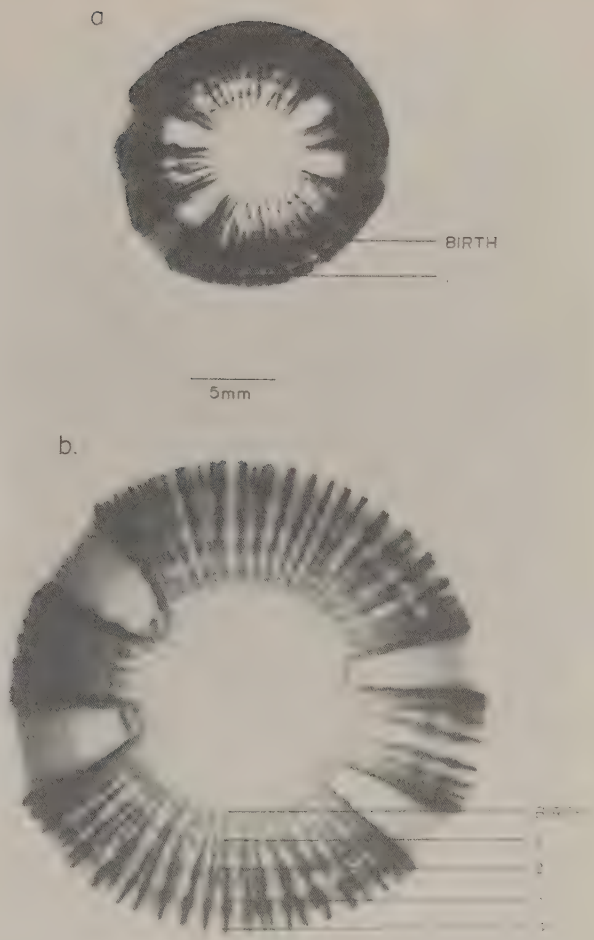

Figure 5.—X-radiographs of centra from common thresher sharks. (a) From a small (1,751 mm TL) free-living male, centrum diameter 15 mm, estimated age 1; (b) from an adult (unknown sex) which measured 3,349 mm TL, centrum diameter 28 mm, estimate age 4.

sharks (Fig. 6). Thus, in future studies, back calculation could prove useful in generating growth curves.

The von Bertalanffy growth curve for the 143 common thresher sharks we aged, which ranged between 360 and 5,733 mm TL, rose gradually and began to level toward the estimated asymptotic length (L_∞) of 6,509 mm TL for both sexes combined (Fig. 7). Females were estimated to reach a longer length (6,360 mm TL) than males (4,927 mm TL). The two oldest fish aged had 15 bands and measured 5,102 and 5,389 mm TL, and the youngest were eight embryos ranging between 360 and 1,605 mm TL, having no bands. Unfortunately, sexes were unknown for most of the fish examined because they were taken from fish markets and had already been cleaned.

The combined asymptotic length from the von Bertalanffy growth curve was 6,509 mm TL, which is only 14% smaller than the maximum reported length (7,600 mm TL), and within the size range of the commonly occurring largest specimens collected in the Pacific (Strasburg 1958; Hart 1973). Using our growth curve, a fish at the asymptotic length of 6,509 mm TL would be close to 50 yr old. Using this approach is questionable, because there are problems associated with the von Bertalanffy growth model, and because we have not collected any specimens near this size. Thus, the maximum age attained by the common thresher shark can be hypothesized, but remains unknown.

Our estimate of size at birth, derived from the von Bertalanffy growth model (1,580 mm TL), was slightly higher than

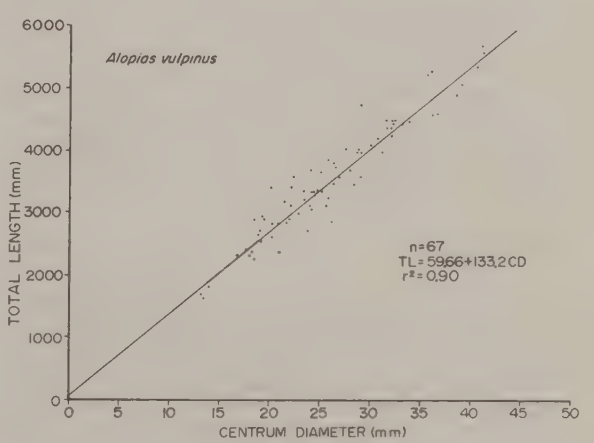

Figure 6.—Regression of the caudal centrum diameter and total length of 67 common thresher sharks.

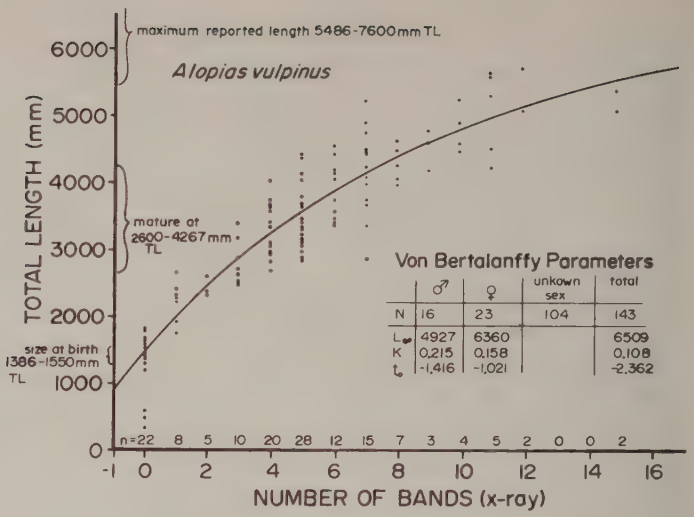
maximum reported length 5486-7600mm TL
Alopias vulpinus
TOTAL LENGTH (mm)
mature at 2600-4267mm TL
size at birth 1386-1550mm TL
Von Bertalanffy Parameters
unkown sex
total
N 16 23 104 143
L∞ 4927 6360 6509
K 0.215 0.158 0.108
t0 -1.416 -1.021 -2.362
n=22 8 5 10 20 28 12 15 7 3 4 5 2 0 0 2
NUMBER OF BANDS (x-ray)

Figure 7.—Von Bertalanffy growth curve for 143 common thresher sharks collected in California waters and aged using X-radiography. Dots represent individuals of both sexes, and von Bertalanffy parameters for males, females, and the total sample are given in the insert. References used for size at birth, size at maturity, and maximum reported size are given in text.

reported smaller sizes of free-living young, which can be as small as 1,168 mm TL (Bigelow and Schroeder 1948), and range up to around 1,500 mm TL (Hixon 1979). One explanation for this difference is that our ageing technique is not precise enough to distinguish time intervals smaller than 1 yr. The mean size of the youngest age class is represented by a single size mode (Fig. 4) and several other mean sizes of younger age classes correspond to size modes, even though our sample was relatively small and nonrandom.

Common thresher shark females range in length at first reproductive maturity from 2,600 mm TL in the Indian Ocean (Gubanov 1978), 3,150 mm TL in Pacific waters (Strasburg 1958), 4,267 mm TL in the Atlantic (Bigelow and Schroeder 1948), and 4,625 mm TL off southern California (Bedford[4]). Bedford (footnote 4), using clasper length versus total length information, estimated that males off southern California first reach maturity at about 3,330 mm TL. These three lengths at first maturity represent sharks which we estimated to range between 3 and 8 yr old (Fig. 7). Using our asymptotic length of 6,509 mm TL, common thresher sharks apparently mature at a size that is between 39 and 71% of this length, which conforms with Holden's (1977) generalization of 60 to 90%. However, if we use the maximum reported size of 7,600 mm TL, these sharks mature at between 34 and 61% of their maximum length. Using age at first maturity versus projected oldest age, the figures would be much lower, reaching maturity at between 6 and 16% of their life span. An increased number of observations on older and larger sharks need to be obtained before a more definitive statement can be made.

Shortfin Mako Shark

Few specimens (50) of the shortfin mako shark were available from the commercial catches between 1978 and 1982 and museum collections, the smallest being a free-living 900 mm TL male and the largest a 3,210 mm TL female (Fig. 8). Although this size range does not include the largest individuals reported worldwide (3,962 mm TL; Bigelow and Schroeder 1948; Roedel and Ripley 1950), nor the largest individual found off California (3,507 mm TL; Applegate 1977), it is representative of the normal size range off California (2,134-2,438 mm TL; Roedel and Ripley 1950).

As with thresher sharks, both age determination techniques enhanced bands, but the X-radiography technique was used to age shortfin mako sharks in this study, because it was faster (Cailliet et al. 1983). Most X-radiographs of centra from shortfin mako sharks were easily assigned ages (Fig. 9), but occasionally outer bands were difficult to discern. We also prepared and read other vertebrae from difficult specimens with silver nitrate for collaboration.

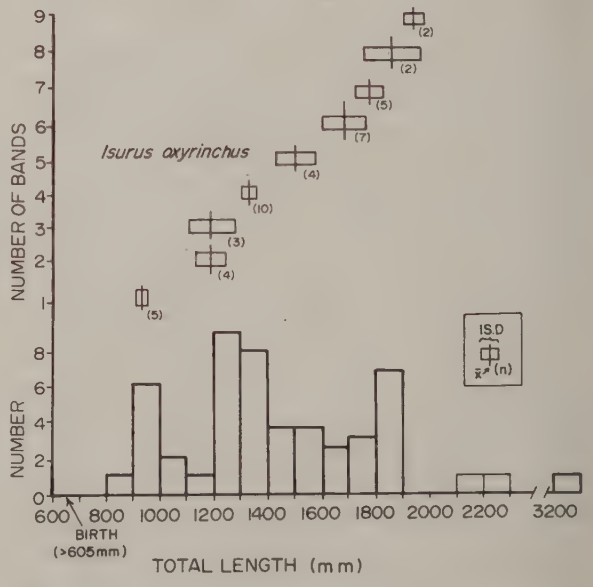

Figure 8.—Size-frequency histogram of shortfin mako sharks collected from California waters and used for age determination. Means (vertical lines) and standard deviations (horizontal bars) of the lengths of all fish placed in a single-age category are shown above the pertinent size-frequency axis. Sample sizes are in parentheses.

[4]D. Bedford, Biologist, California Department of Fish and Game, 245 West Broadway, Long Beach, CA 90802, pers. commun. 1982.

Figure 9.—X-radiographs of centra from shortfin mako sharks. (a) From a small (920 mm TL), free-living male, centrum diameter 11 mm, estimated age 0+; (b) from an adult female (2,110 mm TL), centrum diameter 26 mm, estimated age 6.

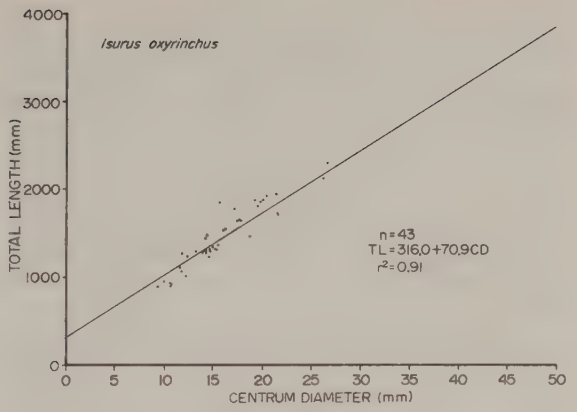

Figure 10.—Regression of centrum diameter and total length of 43 shortfin mako sharks.

A significant ($r^2 = 0.91$; $P < 0.01$) linear relationship was found between total length of shortfin makos and the diameter of their centra (Fig. 10). Thus, in future studies of Pacific shortfin mako sharks, back calculation, a technique used on Atlantic shortfin mako sharks by Pratt and Casey (1983), will be possible.

The von Bertalanffy growth curve for the 44 shortfin mako sharks we aged demonstrates a gently sloping curve which levels off at an asymptotic length of only 3,210 mm TL (Fig. 11). The oldest fish was estimated to have 17 bands and was our largest individual (3,210 mm TL), exactly the same length as our estimated asymptotic length. This age estimate indicates that this specimen was considerably younger than the von Bertalanffy growth model predicted. In addition, the estimated asymptotic length is only 9% less than the the maximum California reported length of 3,507 mm TL (Applegate 1977), but

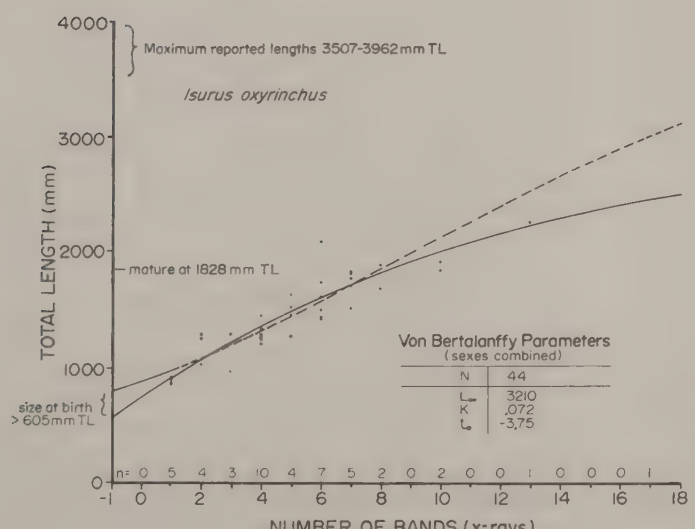

Figure 11.—Von Bertalanffy (solid line) and logistic (dashed line) growth curves for 44 shortfin mako sharks collected in California waters and aged using X-radiography. Sexes were combined due to small sample size, and von Bertalanffy parameters are for all 44 specimens. References used for size at birth, size at first maturity, and maximum size are given in text.

is 16% less than the largest Indian Ocean specimen (3,800 mm TL; Gubanov 1974) and 19% less than the maximum world size of 3,962 mm TL (Bigelow and Schroeder 1948; Roedel and Ripley 1950; Miller and Lea 1972). Using the logistic growth equation on the same data produces a different curve and a more reasonable estimate of asymptotic length of 4,081 mm TL (Fig. 11), which is only 3% higher than the reported maximum sizes worldwide. The differences between the curves produced by these two growth models may be due to their differential sensitivity to the ages assigned to the smallest and largest individuals; hence, increased samples of these size classes should clarify the shape of the curves.

The only other study of growth in the shortfin mako shark was performed by Pratt and Casey (1983) and suggested a growth rate for Atlantic shortfin makos that was approximately twice as fast as our data suggest. They based their growth information on size frequency (N = 848) and length-month analysis (N = 175), tag-recapture length estimates (N = 27), and back calculations from band counts in vertebral centra (N = 109). Their growth rates, based upon size frequency analysis for smaller (and therefore younger) size classes, conformed more to our estimated growth curve than for the larger (older) size classes. This is probably due to the difficulty in accurately and precisely delineating size modes in larger fishes, which are often comprised of several age classes (Ricker 1979).

Pratt and Casey (1983) reported an overall mean growth rate of 25.3 cm/yr based on their tag-recapture analysis of 27 shortfin mako sharks from the northwest Atlantic but considerable variation of this estimate was evident (SD = 41.2). This Atlantic shortfin mako growth rate was about twice as fast as the growth of this species we describe from California waters (overall mean from mean total lengths at successive ages, Fig. 8, of 12.9 cm/yr, SD = 8.5). This discrepancy could be related to differences in habitat and environmental conditions or differences in sample size or ageing methodology used in each of these studies; however, it is interesting to note that the growth rate reported by Pratt and Casey (1983), based on their back calculations from counts of bands on centra, would be similar to ours if each pair of bands from their fish were interpreted as an annual event.

Our estimates of size at birth, derived from either the von Bertalanffy or the logistic growth curves, agree with the scanty information available about the smallest, free-living shortfin mako sharks (Fig. 11). Garrick (1967) examined two embryos that were 605 mm TL, and one free-living male which measured 705 mm TL, while the smallest free-living shark examined by Gubanov (1978) was 900 mm TL, and that by Strasburg (1958) was 1,251 mm TL. The mean size for 1-yr-old shortfin mako sharks corresponds to the first size mode in sharks collected, while the next 3 yr correspond to a single mode (Fig. 8). Extrapolation to the age at which these sharks reach asymptotic lengths estimates longevity to be about 45 yr, based upon both growth models.

Shortfin mako sharks reportedly do not mature until they reach a length of 1,800 mm TL (Gubanov 1978) to 1,828 mm TL (Bigelow and Schroeder 1948), which corresponds to our estimated age of 7-8 yr (Fig. 11). Thus, shortfin makos reach first maturity at a size that is apparently only 56 to 57%, or 44 to 45% of the asymptotic lengths estimated by the von Bertalanffy and logistic growth models, respectively. They reach first maturity at a size that is only 51% of the maximum length reported off California, and 45% of the maximum world size, both below Holden's (1977) generalization. If age at first maturity and the predicted age (45 yr) at which asymptotic length is reached are used, the figures would be much lower, with predicted maturity at between 15.5 and 17.8% of their life span.

LIFE HISTORIES

Many problems arise in estimating age and growth patterns of large and mobile organisms, which need to be considered in relation to our findings (Brothers 1983). It is difficult to obtain sufficient samples of all size classes, due to the high cost and the time involved. The size and activity of these fishes make them difficult to measure accurately. Because market fish are often used and are usually cleaned, a conversion from an available shorter dimension, such as the distance between origins of both dorsal fins to our standard unit of measure (TL), may cause some errors in estimating size. However, the techniques we have developed and applied to delineate bands in centra of these three species have provided consistent results, and the resultant growth curves are generally supported by size at birth and asymptotic or maximum length information. A major objective is to understand the periodic nature of the band formation in shark centra. Even where tag-recapture length information is available, interpretations are often limited by the accuracy and precision of the measurements (Pratt and Casey 1983). There are promising techniques available (Cailliet et al. 1983) which, when applied to these species in more large-scale and comprehensive sampling programs, may increase our understanding of their growth processes.

Our preliminary findings on age and growth, coupled with the literature on size and reproductive characteristics, indicate that these three pelagic species, which often occur together in coastal areas around the world, differ in their life histories. The blue shark is generally smaller than either the shortfin mako or the common thresher sharks. Because the upper lobe of the common thresher shark's tail comprises almost half of its total length, it is more appropriate to compare the weight of these fishes. The common thresher and shortfin mako sharks range up to about 454 kg maximum (Bigelow and Schroeder 1948; Applegate 1977), while the largest blue shark ever taken probably weighed about 181 kg (Bigelow and Schroeder 1948; Strasburg 1958). Considering tail length, size at birth also exhibits a similar trend. Blue sharks are between 340 and 530 mm TL at birth, while shortfin makos range between 705 and 900 mm TL, and common threshers 1,386 and 1,552 mm TL (Bigelow and Schroeder 1948; Garrick 1967; Gubanov 1978; Pratt 1979). Size at first maturity, which varies considerably among individuals, appears to be similar for all three of these species. The blue shark ranges in length at first maturity from 1,800 to 2,500 mm TL, while the values for common threshers and shortfin makos are 2,600 to 4,265 mm TL, and 1,800 to 1,828 mm TL, respectively (Bigelow and Schroeder 1948; Gubanov 1978; Pratt 1979). Relative to ultimate maximum size or age, the blue shark reaches maturity later than either the common thresher or shortfin mako sharks.

There is an apparent trend for fecundity to be less in the larger of these three species, although the available information on their reproduction is relatively sparse. Blue shark fecundity estimates range from 23 to 135 per female (Tucker and Newnham 1957; Gubanov 1978; Pratt 1979), while the best estimates for shortfin makos are between 2 and 10 (Bigelow and Schroeder 1948; Gubanov 1978), and for common

threshers are between 2 and 4 (Bigelow and Schroeder 1948; Strasburg 1958; Hixon 1979). There is very little information about the gestation period for pelagic elasmobranchs. Pratt (1979) estimated that blue shark embryos reach full term in 9 to 12 mo. Our growth curve supports this contention. Virtually nothing is known about the gestation period of the other two species, but Bedford (footnote 4) has estimated gestation to be 9 mo long in common thresher sharks off southern California.

In conclusion, our preliminary data and the available literature indicate that these three pelagic sharks attain large sizes, have gradual growth rates, long life spans, and relatively low but variable fecundities. Therefore, as first postulated by Holden (1973, 1974, 1977), it is quite possible that this combination of life-history traits could make these species susceptible to overfishing. However, this conclusion may be countered by our estimate of a relatively early age of first reproductive maturity. More extensive samples of all sizes over a wider geographical range, an equal representation of sexes, and more detailed analysis of age, growth, and reproduction need to be conducted before definitive statements can be made about the life histories of these species. Also, more needs to be known about their population abundance, distributions, and migration patterns. Only when this information is available will we be able to accurately predict the future of these fisheries, and perhaps satisfactorily manage them.

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SUMMARY PAPER

Age and Growth of the Sandbar Shark, *Carcharhinus plumbeus*, from the Western North Atlantic[1]

JOHN G. CASEY, HAROLD L. PRATT, JR., and CHARLES E. STILLWELL[2]

The sandbar shark, *Carcharhinus plumbeus*, is cosmopolitan in distribution (Garrick 1982). Much of what is known about its life history and biology in the western North Atlantic is summarized in a monograph on the species by Springer (1960). He reported that the growth rate of the sandbar shark was not known and discussed the possibility of 2 to 3 yr to maturity based on length-frequency data. Wass (1973) reported age to maturity to be between 3 to 13 yr for Hawaiian specimens, and Lawler (1976) provided age estimates up to 23 yr based on limited examination of rings in the vertebrae. However, none of these studies validated the formation of vertebral rings as annual marks. We report age and growth estimates of the sandbar shark based on interpretations of growth rings in the vertebrae (including aquarium-held sharks), results of tag and recapture data, and analysis of length-frequency data.

Our main source of sharks for tagging, vertebral samples, and length-frequency data came from a commercial shark fishery at Great Machipongo, Va., (1965-69) and from research cruises and sport fishing tournaments in the Mid-Atlantic Bight (1965-81). All lengths are reported as fork lengths (FL) unless otherwise noted. (Total length [TL] was converted to fork length using the regression FL = 0.8265 TL + 1.3774, $r^2 = 0.99$, $N = 4{,}250$.)

The 15th through 20th vertebrae were sampled for age analysis. The vertebral sample was cleaned and preserved in 10% Formalin[3] or Bouin's solution. The 19th and 20th vertebrae were processed histologically and one (usually the 20th) was sectioned for interpretation. The histological technique used is a standard process for the preparation of calcified material (Humason 1972). After embedding, vertebrae were sectioned longitudinally with a sledge microtome to obtain 80-100 μm sections from the center of the centra. Growth bands (opaque) on sections were counted and the distance from the core to the outer edge of each band was measured using an ocular micrometer in a dissecting microscope at 10× magnification with transmitted light. Vertebral measurements were used to back-calculate fork length at estimated ages.

From 1963 through 1981, about 5,000 sandbar sharks were tagged with cattle ear tags (rototags) or with modified "M" dart tags (Davies and Joubert 1967) and released as part of the NMFS Co-operative Shark Tagging Program. Of these, 220 were returned and analyzed. We measured and tagged a 92 cm FL male sandbar shark in 1974 at the New England Aquarium that we subsequently measured in 1979 and again when it died in 1981. We measured a second shark (a 137 cm FL female) there in 1979 that died in 1981.

Analyses of rings in the vertebrae of 475 individuals were used to prepare growth curves. Vertebrae from seven tagged sharks at liberty for up to 8 yr and from two sharks held in captivity for up to 6+ yr were used to verify annual marks in vertebrae of sharks to age 14+ yr. Length measurements at release and recapture were made by us on six of the recaptured individuals from which vertebrae were obtained. Vertebrae from all recaptured sharks displayed one distinct growth mark per year for each year at liberty. The vertebrae from sharks maintained in captivity also showed one mark per year between the time they were measured and died (1+ to 6+ yr). Comparative growth curves are shown in Figure 1.

Growth data from 220 tagged sharks at liberty for up to 17 yr agreed closely with annual growth rate estimates based on interpretation of bands on vertebrae. Modes in length-frequency distributions were good indicators of age groups for the first 5 yr and concur with age estimates based on vertebrae (Fig. 2).

The oldest male sandbar shark aged in this study was 15 yr old at 154 cm FL, the oldest female was 21 yr old at 204 cm FL. Estimated age to maturity is 13 yr for males, 12 yr for females. The growth rate is similar in both sexes, although females reach a larger adult size. The mean growth rate from vertebral back calculations was 7.3 cm/yr (SD = 4.5). The mean growth rate derived from tagging data for combined sexes was 5.2 cm/yr (SD = 2.7). Tag returns indicate sandbar sharks may live for over 30 yr. Vital von Bertalanffy parameters are: Male L_∞ = 257 cm FL, k = 0.050, t_0 = −4.5 yr; female L_∞ = 299 cm FL, k = 0.040, t_0 = −4.9 yr.

The strengths and weaknesses of the von Bertalanffy growth formula (VBGF) have been examined by several authors including Knight (1968), Ricker (1975), Roff (1980), and Pauly (1981). Despite its shortcomings, the VBGF appears to adequately describe the growth of the sandbar shark from birth to age 18 yr. The t_0 values are high for the sandbar shark compared with the known gestation period of about 1 yr (Springer 1960). Intrauterine growth is so much faster than subsequent growth that unrealistic t_0 values result. The L_∞ values also appear high since the maximum reported size for the sandbar

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[2]Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA, Narragansett, RI 02882-1199.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

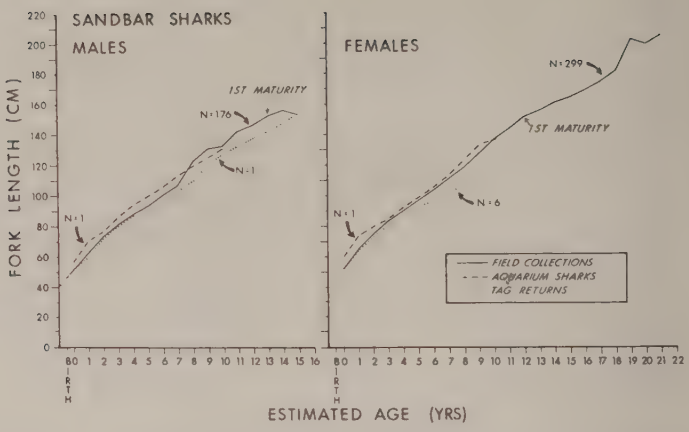

Figure 1.—Growth estimates from back calculation of annual marks in vertebrae of sandbar sharks from three sources: 1) Aquarium sharks, two captive sharks held in the New England Aquarium for several years; 2) tag returns from seven tagged sharks; and 3) field collections from 475 sharks.

shark is 212 cm FL (a result of this research). Pauly's (1981) modified VBGF gave more realistic L_∞ values with almost identical size at estimated age values shown in Figure 3. These findings lend support to Pauly's contention that a generalized VBGF should be employed, at least for the sandbar shark.

Growth curves for individual sandbar sharks consistently show similar periods of fast and slow growth. In the first 2 to 3 yr, growth is relatively fast, followed by a slow down between estimated ages 4 through 9 (90-130 cm FL), after which the growth rate increases for the next few years, then appears to slow and remain constant for the remainder of life. The von Bertalanffy equations do not predict the slower growth between the range 90-130 cm FL. A fifth degree polynomial regression provided the best fit to the actual back-calculated values shown in Figure 1 (field data). The reduced growth rate in juveniles coincides with the size range at which they move offshore, away from the estuarine summer nursery grounds. Since the shift to an offshore life phase occurs before maturity, the change in growth rate may be related to a limiting factor such as oxygen (as discussed by Pauly 1979), or a change in food habits. Gruber and Stout (1983) reported similar growth and offshore movement in adult lemon sharks, *Negaprion brevirostris.* They speculated that growth of the lemon shark may also be affected as a consequence of habitat change and/or related changes in food availability. Predictive growth parameters for the sandbar shark using a generalized von Bertalanffy growth formula (Pauly 1981) and a polynomial regression are provided in our full length manuscript (footnote 1).

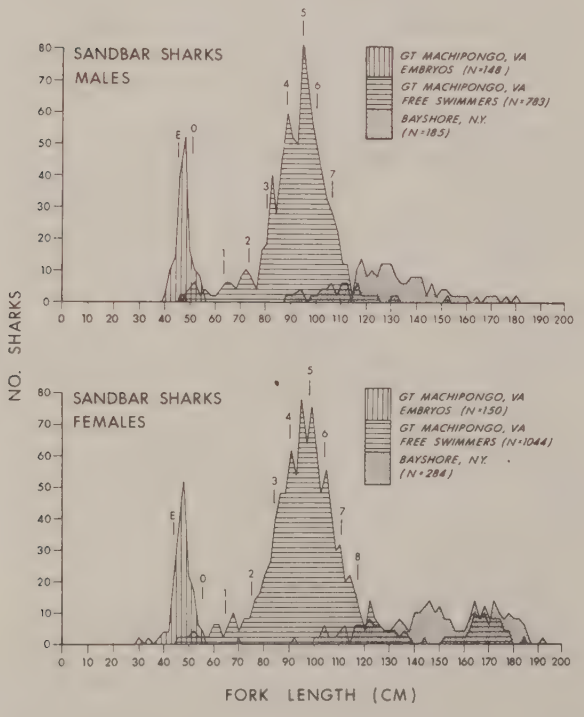

Figure 2.—Length-frequency distributions (i = 2 cm) of sandbar sharks examined in June at Great Machipongo, Va., 1965-69; and Bay Shore, N.Y., 1965-80. The numbers above the frequency peaks indicate the mean back-calculated size at estimated age from analysis of vertebrae.

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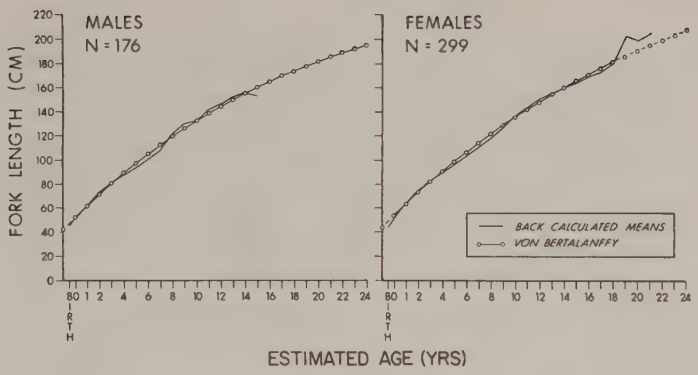

Figure 3.—Von Bertalanffy growth curve fit to back-calculated estimates of fork lengths at estimated age: Males, $L_\infty = 257$ cm FL, $k = 0.0501$, $t_0 = -4.5$ yr, $N = 176$; females, $L_\infty = 299$ cm FL, $k = 0.040$, $t_0 = -4.9$ yr, $N = 299$.

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Biological Materials for the Study of Age and Growth in a Tropical Marine Elasmobranch, the Lemon Shark, *Negaprion brevirostris* (Poey)

SAMUEL H. GRUBER and ROBERT G. STOUT[1]

ABSTRACT

A total of 1,526 lemon sharks, *Negaprion brevirostris*, were examined to estimate age and growth by analyzing growth zones on histologically stained and tetracycline-treated vertebral centra, and long-term growth in the laboratory and the field. A newborn 48 cm precaudal length (PCL) lemon shark grows at a rate of about 0.3 mm/d and reaches 60 cm PCL after 1 yr. One year old lemon sharks of this size can have as many as three growth zones on their vertebral centra. Overall, results demonstrated that the lemon shark is a slow-growing, late-maturing, long-lived tropical shark reaching maturity in no less than 12 yr. This finding stands in contrast to earlier reports of rapid growth and maturity in 1 to 3 yr. A von Bertalanffy growth curve based on size at birth, gestation period, and maximum size overestimated growth in the field by about 50%.

INTRODUCTION

Any model of the functioning of an ecosystem must account for the effects of higher predators that can exert both stabilizing and oscillating influences on their environment (Volterra 1928; Hassell 1976). In the marine ecosystem, the influence of one potentially important group—the sharks—remains largely unexamined (Strasburg 1958; Moss 1972; Soucie 1976; Gruber 1982). This is because they are swift, shy, wide-ranging, live in a concealing medium, and are difficult to keep. Thus, few details are known about their life history (Holden 1974; Gruber and Myrberg 1977; Brett and Blackburn 1978).

In an attempt to fill this gap, we have gathered laboratory and field data on the autecology of the lemon shark, *Negaprion brevirostris*, with the overall goal of producing a model of production for this species. The production rate of the individual can then be extrapolated to the population if estimates of abundance, mortality, and age and growth are known (Le Cren 1974). However, the age structure of most elasmobranch populations remains unknown because they have not been systematically exploited, skeletal structures traditionally used for ageing teleosts (i.e., scales and otoliths) are not suitable for ring counting, and ageing tropical species may be less reliable since uniform growing seasons do not favor the formation of periodic marks on skeletal structures. In addition, most sharks studied to date apparently grow slowly, with the result that length-frequency plots are virtually useless for separating age classes beyond the second or third year.

More recently, studies of age and growth in sharks have provided growth curves using concentric marks on the face of the vertebral centrum (Hoenig 1979). In several cases (Aasen 1963; Tanaka and Mizue 1979), counts of these growth features were combined with length-frequency and other biological data leading to the suggestion that vertebral circuli were formed annually, thus providing a good estimate of yearly growth and age-at-size. Yet, the only direct proof that the vertebral growth marks of an elasmobranch are formed annually is that of Holden and Vince (1973), who used tetracycline as an internal time-mark to validate the age of a temperate skate.

In this paper, we report on age and growth of the lemon shark based on laboratory rearing, field tagging, catch data, and a preliminary examination of histologically stained and tetracycline-treated vertebrae.

MATERIAL AND METHODS

Between June 1979 and September 1982, we collected 1,526 lemon sharks from 45 to 180 cm precaudal length (PCL) by rod and reel fishing, longlines fixed to the bottom, or by cast netting. Specimens were taken from three sites: 1) Sharks larger than 150 cm PCL were taken from the Marquesas Islands southwest of Key West, 2) juveniles (40-70 cm PCL) were collected east of Matecumbe Key, Fla., in Everglades National Park, and 3) about 75 specimens of various sizes were measured at Bimini, Bahamas. Usually precaudal length (i.e., snout tip to anterior edge of precaudal pit) and total length were measured to the nearest 0.5 cm. When possible, sharks were weighed to the nearest 10 g.

Vertebral Staining and Measurement

Our methods to estimate the age of lemon sharks include the counting and interpretation of periodic growth zones on vertebrae through tetracycline marking and histological staining; the analysis of length-frequency distributions; regression analysis of length vs. vertebral dimensions; and observations of long-term growth in the laboratory and in field tagging experiments. To visualize growth zones, whole vertebral columns were excised from 21 lemon sharks (48 to 180 cm PCL) and excess tissue, including neural and hemal arches, was removed from each column. The number of vertebrae was counted and the vertebral column air dried for storage.

We used the histological technique of La Marca (1966), which consisted of separating the individual dried centra and soaking

[1]University of Miami, Rosenstiel School of Marine and Atmospheric Science, Division of Biology and Living Resources, 4600 Rickenbacker Causeway, Miami, FL 33149-1099.

them in a 0.1% solution of sodium hydroxide. This softened the notocordal remnants and connective tissue which were removed and discarded. Whole vertebrae were then immersed for at least 24 h in a stain consisting of a saturated aqueous solution of alizarin red S and 0.1% NaOH in the ratio of 1:9. After staining, specimens were washed in running water and differentiated in a 3% hydrogen peroxide solution. To visualize growth zones in cross section and to explore the internal banding pattern, some vertebrae were cut or ground flat in various planes. This was done by lapping the concave anterior face of the centrum on a glass plate with 600 grit abrasive until it was flat, or by grinding off the entire left half of the centrum until its inner surface was visible at a plane bisecting it into halves. The measurements were usually done on the 45th centrum (counting from the head back). Centra 40 through 50 were prepared in the same manner.

The centrum face took up stain differentially. Under a low-power dissecting microscope (20×) with white reflected light, concentric lines could be distinguished. These numerous lines or "circuli" differed from the background in both texture and density. In centra > 6 mm diameter, the circuli were arrayed together into wider concentric structures which we called bands. Bands could be counted macroscopically because there was a light-colored zone separating zones of intensely stained cartilage. However, the combinations of bands and circuli, especially in the larger centra, introduced some uncertainty in our subjective method of counting. The actual counts of growth bands were done by one recorder and then comparisons of these were made with counts based on a photograph of the specimen. There were no further attempts to reduce or measure subjective counting error (i.e., precision; see verification in Glossary).

Counting back from the head, two measurements of one centrum between no. 42 to 47, from 20 sharks, were taken. The horizontal diameter of the anterior face and the length (i.e., the distance between the anterior and posterior faces) were measured to the nearest 0.1 mm with a vernier caliper. Relationships between the centrum values and precaudal length were determined using regression analyses. These and all statistical inferences in this paper are based on an alpha level of $P = 0.05$.

Tetracycline Treatment

When tetracycline was shown to combine in vivo with bone calcium (Milch et al. 1957), providing a relatively permanent fluorescent mark, it became apparent that this antibiotic held great potential for validating the time scale on any regularly occurring growth pattern of a mineralized structure (Weber and Ridgway 1962). Tetracycline marking has been used only infrequently over the years, but where it has been successfully applied in connection with ageing studies, it has provided one of the few direct methods for validating the time between formation of growth rings. Therefore, we injected each one of our tagged sharks with tetracycline hydrochloride (Achromycin[2] Lederle Laboratories) at a rate of 12.5 mg/kg. Sharks held in the laboratory were also given tetracycline.

The antibiotic was stored in sterile, air-tight vials containing 100 mg of tetracycline HCl, 40 mg Procaine HCl, 47 mg magnesium chloride, and 250 mg ascorbate. This package, intended for intramuscular injection, contained instructions stipulating that the contents of the vial should be mixed with 2 cc of distilled water to a final concentration of 50 mg/ml. Tetracycline was injected with a sterile syringe 1 cm deep into the shark's epaxial musculature just posteriolateral to the first dorsal fin. Preliminary observations in the laboratory indicated that within a range of 5-100 mg/kg, dosage was not critical for ultimately distinguishing the fluorescent bands. Additional studies on dose level, uptake rates, and overall effect of tetracycline injections on captive sharks are underway.

By the time of the analysis (December 1981), we had obtained tetracycline-treated centra from 12 lemon sharks (45-80 cm PCL). Time from injection to death varied between 5 and 532 d. Only three of these had been tagged and recaptured. The remaining nine were studied in captivity.

Tetracycline-treated centra were removed, dried, cut, polished (see Vertebral Staining and Measurements), and photographed both under white and ultraviolet light. For the fluorophotographs, ultraviolet light was isolated from the spectrum of a 100 W mercury-arc flood lamp (Westinghouse H38-4JM Par 38) by passing the lamp's output through a KOP no. 41 ultraviolet filter. The lamp and filter were installed in a "Blak-Ray" long wavelength ultraviolet lamp housing (Blacklight Eastern Corp.) and the lamp was run with a "Woods Light" power supply of 100 W, 2A (Ultraviolet Products).

Tetracycline has a strong absorbance band at a wavelength of 353 nanometers (nm) and reemits at wavelengths > 515 nm (Undenfriend et al. 1957). The result is a brilliant golden band of intense fluorescence wherever tetracycline is bound to calcium in the tissue (Fig. 1).

Permanent records of tetracycline uptake in the vertebral centra were made by photographing the dried specimens under ultraviolet light with a Nikon 35 mm camera coupled to a 55 mm Micro-Nikkor macrolens and bellows extension. The photofluorographs were taken through a Wratten W 2A filter. This light-yellow filter transmits the tetracycline fluorescence relatively better than the bluish autofluorescence of the cartilage, thus enhancing contrast between sample and background.

For comparison, photographs of an adjacent alizarin-stained centrum and a tetracycline-treated fluorescent centrum were printed at the same magnification and a composite of both was made. Thus, it was possible to objectively relate the position of a growth zone to the fluorescent tetracycline ring as shown in Figure 2. Further detailed measurements were made by projecting the original transparencies and measuring dimensions of the enlarged image.

Growth Studies

Indoor aquarium, controlled experiment.—Growth of lemon sharks was determined in three separate experiments. During the course of an evaluation of food intake (Longval et al. 1982), six lemon sharks (average 55.6 cm PCL) were held in three 200 l aquaria under carefully controlled conditions of temperature, water quality, and illumination for 100 d. These animals were weighed and measured at the beginning and end of the study and fed blue runner, *Carynx chrysos*, fillets to satiation twice per day over a 95-d trial. The amount of food offered to each shark was preweighed so that it was possible to calculate growth as well as the efficiency of conversion of food

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

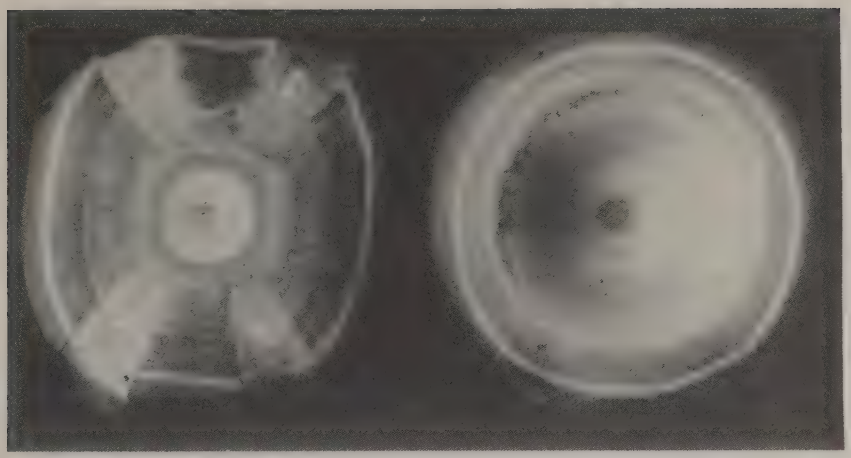

Figure 1.—Fluorophotographs of two tetracycline treated centra from lemon shark no. 17. This 70 cm precaudal length (PCL) male was captured, tagged, and released in April 1980, recaptured 335 d later in March 1981, at 78 cm PCL, and returned to the laboratory where it lived another 330 d. At death, in January 1982, it had grown to 82 cm PCL; its centrum diameter had increased from 4.10 to 10.25 mm. The tetracycline fluorophor is represented by a white ring encircling the centrum. In the left photograph, the concave front face of the centrum has been lapped flat on a glass plate with no. 600 grit. In the right photograph, only the edge has been lapped to enhance the tetracycline ring.

Figure 2.—Composite photograph of two centra from a 59 cm PCL female lemon shark (no. 16), which was captured, tagged, injected with tetracycline, and released in June 1980 and recaptured 532 d later in December 1981. Centrum diameter is 7.2 mm and growth rate was 0.3 mm/d. The upper half is centrum no. 44 which has been ground flat as described in the text, stained with alizarin red S, and photographed in white light. The lower half is centrum no. 45 which has been ground flat and photographed in ultraviolet light. The tetracycline ring is medial to the growth marks showing that the first growth band does not form until some months after birth. This specimen possessed three growth bands at death.

into shark tissue. In this study, growth was expressed either as an increase in total length or an increase in wet weight.

Outdoor pond, uncontrolled experiment.—In a second study, 30 specimens (average 51.4 cm PCL) were held in an outdoor pond for 89 d. Nine of these were subsequently moved to a second pond and kept for another 120 d. Sharks were weighed and measured once at the beginning of the study and then up to three more times during the maximum 220 d of captivity. Most, however, were measured on the day the study began and again 89 d later. This group of sharks was fed whole threadfin herring, *Opistonema oglinum*, to satiation twice each day.

Field tagging study.—In a third study, sharks were captured for tagging by cast net and transported to a collection center where they were allowed 24 h to recover. Each specimen was then weighed, sexed, and measured (TL, PCL) before marking. Marks included freeze bands, mini-rototags in the first dorsal fin, plastic dart tags in the back muscle, internal tags surgically implanted in the coelom, and tetracycline intramuscularly injected at 12 mg/kg. Approximately 1,500 sharks have been marked and released in this manner since 1979. Over 70 recaptures (5%) have been reported but reliable growth data were obtained from < 20 sharks. Many of the recaptured sharks were released again as part of our ongoing program. These returns were compared with those of 16 lemon shark returns from a similar, unpublished marking experiment conducted by Starck[3] in 1967.

[3]W. A. Starck II, Marine Biologist, PMB 1, Damtree, Queensland, 4873 Australia, pers. commun. 1981.

Monthly catch records.—An indirect measure of growth was obtained by randomly selecting a stratified sample of eight sharks for each month of the year. The individual length for each shark captured for each month was recorded; thus, growth of the year class could be followed.

Von Bertalanffy growth curve.—Finally, we have produced a provisional von Bertalanffy curve based on Holden's (1974, 1977) concept that parameters can be calculated from a knowledge of intrauterine growth combined with the maximum size of the shark. Holden thus reorganized the basic von Bertalanffy (1960) equation as follows:

$$l_{t+T} - lt = (L - l_t)\,(1\text{-}e^{kT}) \qquad (1)$$

where l_{t+T} = length at birth = 60 cm TL (47 PCL),
l_t = length at fertilization = 0 cm,
T = gestation period = 12 mo,
L = maximum size = 368 cm TL (281 PCL), and
k = the growth constant.

We have estimated the parameters of this equation, calculated k, and produced a curve using a iterative solution given by Allen (1966). We also plotted growth data from recaptured tagged sharks and age estimates from growth marks on the vertebral centra to see how well the theoretical curve predicted some field results.

RESULTS

Growth Bands on the Centrum

In a subsample of 24 sharks (40.3-131.2 cm PCL), the count of body vertebrae averaged 117 (range 110-121). Of these, the 40th through 50th were among the largest vertebrae and so the 45th centrum was chosen for analysis. Results of alizarin red S staining (Fig. 3, Table 1) demonstrated that the calcified centra of lemon sharks show concentric growth bands which are very distinct. Of the 21 sharks with stained vertebrae, 6 (48-53 cm PCL) were assigned an age of 0+ according to criteria based on size at birth, growth of recaptured marked sharks, and growth in captivity. The centra of these young-of-the-year had a single growth band with a uniform diameter of 5.3 mm (Table 1). In the larger sharks (55-179 cm PCL, N = 15), the diameter of the first band was also quite consistent, its average value being only slightly higher (5.4 mm). Thus, intrauterine growth and growth in the first year are probably uniform among sharks of different year classes. In a sample of eight larger sharks, the width of the second growth band varied between 0.3 and 0.8 mm. In sharks with more than two bands, the measured intervals varied about 15-fold, giving the impression that later growth may be quite variable. The number of growth bands generally increased with length (Fig. 4). The maximum count was 28 in a 170 cm PCL specimen. However, a 178 cm PCL shark possessed only 17 bands. It is possible that staining artifacts and subjective errors may account for some of this variability. Yet, the overall growth of vertebrae was consistent among sharks. In a sample of 20 specimens (49-182 cm PCL), regression analysis of two vertebral dimensions vs. shark length demonstrated a linear relation between these variables (Fig. 5). The correlation between both measures was significant (r^2 for centrum length = 0.97; r^2 for centrum diameter = 0.99). Thus, the vertebral centrum with its growth bands should be an adequate structure on which to base back calculations of size-at-age.

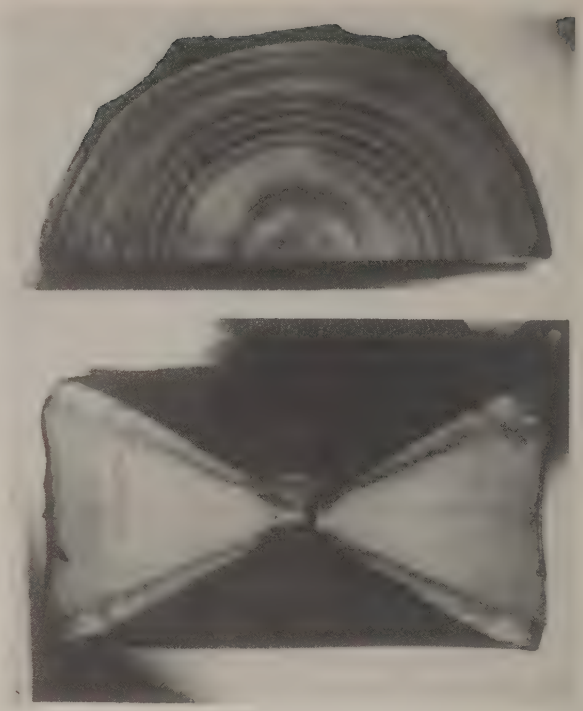

Figure 3.—An alizarin-stained vertebral centrum cut in half in the lateral plane from a female lemon shark (no. 8), 178 cm PCL, captured in the Marquesas, Fla., September 1981. Upper photograph shows the dorsal half of the centrum as viewed posteriorly (i.e., toward the tail). The lower photograph shows a median lateral cross section of the same centrum. The hourglass-shaped structure represents the left and right side of the dorsal half of the centrum cut in half. Seventeen growth bands were counted in this specimen. Calibration is 5 mm.

By comparing length-at-age as indicated by tag returns, it appears that lemon sharks produce up to three growth bands each year (Table 1). For example, a tagged newborn lemon shark recaptured after 1 yr will have grown to about 58 cm PCL. We counted one to three growth bands in centra from sharks of this size. A newborn shark at liberty for 2 yr reaches about 65 cm PCL. According to our counts, a shark of this size has six concentric bands. We estimate that a 4-yr-old 80 to 85 cm PCL lemon shark carries 10 to 12 growth bands. These comparisons do not entirely coincide with our tetracycline validation, which indicates three bands in the first year but possibly only two bands thereafter.

Tetracycline Validation

Intramuscular injection of 12.5 mg/kg tetracycline HCl will produce a distinct fluorescent ring on the growing edge of the centrum of a lemon shark (Table 1, Fig. 1). While the amount of tetracycline bound to the calcified tissue varies with dosage, time for ring formation appears to take about 1 mo. For example, prior to 10 d residence time, there was no evidence of uptake in the centra of three lemon sharks. At 10 d, fluorescence appeared around the notochord of one shark, and by 30 d two specimens showed distinct rings at the edge of the centrum face.

Table 1.—Counts and measurements of the alizarin-stained centra of 21 lemon sharks. Specimens increase in length from left to right.

Shark reference number	15	19	20	18	14	6	3	48	16	1	2	17	4	10	11	9	7	5	13	12	8
Precaudal length (cm)	48	49	49	51	53	53	54	57	59	60	66	80	82	94	98	127	132	133	146	170	178
Number of stained growth bands	1	1	1	1	1	1	2	(−)[1]	3	6	≅6	10	≅10	7	11	13	12	18	13	28	17
Diameter of stained centrum (mm)	6.1	5.5	5.7	5.6	5.8	6.1	6.4	6.9	7.2	7.0	7.4	9.3	9.1	10.2	11.1	16.6	16.8	17.3	18.8	23.2	25.2
Diameter of each stained growth band (mm)	5.0	5.3	5.1	5.5	5.1	5.5	5.6	(−)[1]	5.8	5.6	4.9	5.0	4.1	5.6	5.1	6.0	6.0	5.3	6.0	5.2	6.0
							5.9	(−)[1]	6.2	5.9	5.1	5.5	4.3	6.4	5.8	7.4	6.5	5.8	8.0	5.6	7.6
									6.8	6.0	(−)[1]	5.8	4.6	6.9	6.2	8.6	7.0	6.5	8.8	6.0	9.6
										6.3	(−)[1]	6.6	5.4	7.3	6.7	9.1	8.3	7.0	9.6	6.8	10.4
										6.6	6.7	6.9	(−)[1]	8.0	7.8	10.3	8.8	7.3	10.4	8.0	12.0
										6.7	7.1	7.6	(−)[1]	8.9	8.0	10.9	9.3	9.3	11.6	8.4	12.4
												8.0	7.4	9.3	8.2	11.4	10.3	10.3	12.4	8.8	13.6
												8.4	(−)[1]		8.5	12.0	10.8	10.8	12.8	9.2	14.8
												8.7	(−)[1]		9.8	12.9	11.3	11.5	13.6	9.6	15.2
												9.1	(−)[1]		10.0	13.1	15.0	12.5	14.4	10.4	16.4
															10.7	14.0	15.3	13.0	15.2	11.2	18.4
																14.6	15.8	13.5	15.6	11.6	19.2
																14.9		14.0	17.2	12.0	19.6
																		15.3		13.2	20.0
																		15.5		13.4	21.2
																		15.8		13.6	22.4
																		16.0		14.6	23.2
																		16.5		15.0	
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[1]Stain was poor and band diameter was difficult to measure.

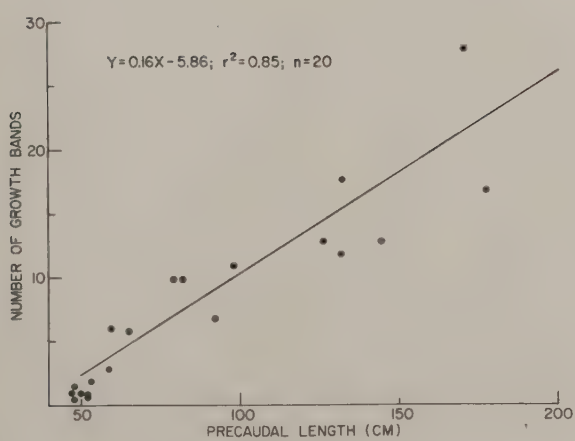

Figure 4.—Relation between precaudal length and number of alizarin-stained growth bands on centrum of 20 lemon sharks. The relationship is linear with a r^2 = 0.85.

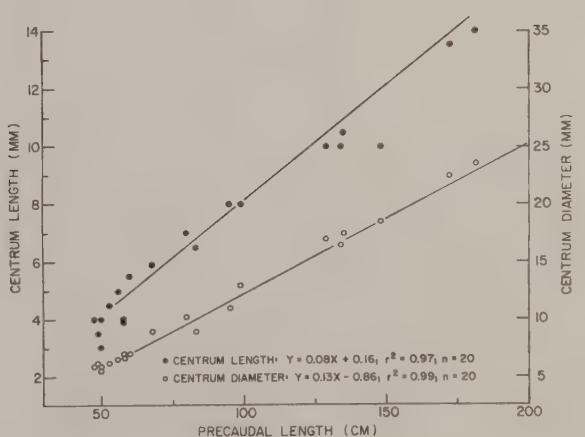

Figure 5.—Relationship between precaudal length and centrum dimensions in 20 lemon sharks. Upper curve (solid circles) represents the anterioposterior length of the 45th centrum; lower curve (open circles) represents the horizontal diameter of the anterior face of the same centrum. The linear fit is significant in both cases (r^2 = 0.97 and 0.99, respectively).

Incidental observations of lemon sharks under ultraviolet light showed intense fluorescence at the site of injection, in the liver, and around jaws, teeth, and all calcified tissues. Fluorescence of the placoid scales gave the shark a speckled appearance under ultraviolet light.

Rate of growth of the vertebral centrum was estimated by measuring the distance between the tetracycline ring and the edge of the centrum, then dividing that value by the time between injection and death. Generally, there was a direct relation between rate-of-body and rate-of-centrum growth. One exception was shark no. 15, which had a high rate-of-centrum growth. Actually, body growth of the 12 tetracycline treated sharks varied over one order of magnitude, reflecting the variable conditions that these sharks were held under.

Most of the specimens in Table 2 were young-of-the-year when first injected. Shark no. 1 was the only shark estimated at 1+ yr. When first injected at 53 cm PCL, this specimen already possessed two growth bands on its vertebral centrum. All other sharks whose centra were stained (except no. 16) had a single growth band. By comparing the position of the tetracycline ring to the first growth band, it became clear that lemon sharks form the first growth mark some months after birth. For example, shark no. 18 was 50 cm PCL when first injected in June 1981. Upon examination of its centrum 220 d later, we found that the tetracycline ring was medial to the first growth band by 0.5 mm. Assuming a centrum growth rate of 2.8×10^{-3} mm/d, the first growth band would have been deposited in November 1981, some 6 to 7 mo after birth. Sharks no. 16 and 19 also deposited their first growth band several months after tetracycline injection.

The only shark that was free in the field long enough to validate the production of growth bands was no. 16. The 48 cm PCL female was captured, marked, and released 16 June 1980. This shark was recaptured 532 d later on 12 February 1982, and killed by the angler. We were notified, measured the specimen, and removed its vertebral column. The shark had grown at an average rate of approximately 0.2 mm/d over the 18-mo period to a final size of 57.3 cm PCL. Tetracycline marking and alizarin staining demonstrated that the centrum had a horizontal diameter of 5.4 mm when the shark was first captured. Assuming continuous unaccelerated growth of the centrum, we estimated that the first growth band was formed in September 1980, 4 to 5 mo after birth. A 1981 winter band at 6.2 mm diameter and fall band were elaborated; the shark died before its second winter. Thus, three distinct growth bands had formed in the 1.5 yr since tagging (Fig. 2).

Growth Rate

Growth data for most sharks were determined by measuring precaudal length. Some sharks had only total length measured. Thus, we have plotted precaudal length against total length to determine if it is possible to calculate one value from another. The result was a significant linear relationship:

$$\text{PCL} = 0.76\ \text{TL} + 2.85\ (n = 71;\ r^2 = 0.99). \qquad (2)$$

Table 2.—Measurements of growth and centrum features of eight lemon sharks treated with 12.5 mg/kg tetracycline and which lived for at least 150 d after treatment. Shark nos. 14, 16, and 17 were tag-recaptures; the rest were kept in aquaria. Growth rates of sharks were arranged to increase from top to bottom.

Shark no. and sex	Time between tetracycline injection and death (d)	Precaudal length at injection (cm)	Growth rate (mm/d)	Centrum growth (μm/d)	Centrum diameter at death (mm)	Diameter of first stained band (mm)	Diameter of fluorescent tetracycline ring (mm)
20 M	239	49	0.04	0.8	5.7	5.1	5.5
18 F	216	50	0.09	2.8	5.6	5.5	5.0
19 M	225	48	0.13	1.3	5.5	5.3	5.2
15 F	155	46	0.26	6.5	6.1	5.0	5.1
16 F	532	48	0.30	3.4	7.2	5.8	5.4
48 M	455	46	0.33	4.5	6.9	poor stain	4.9
14 F	252	48	0.40	1.0	5.8	5.1	5.6
1 F	224	53	0.45	4.9	7.0	5.6	5.9

Table 3.—Food intake and growth of six lemon sharks held in the laboratory, under controlled conditions, and fed to satiation daily for 95 d.

Shark no. and sex	Estimated precaudal length at start of experiment (cm)	Total food intake over 95 d (g)	Increase in weight over 95 d (g)	Increase in length over 95 d (cm PCL)	Coefficient of utilization for growth[1] (%)
1 M	60	6,240	1,587	11.0	25.43
2 M	57	5,410	1,372	10.5	25.36
3 F	56	3,280	348	1.5	10.61
4 F	55.5	4,030	588	2.0	14.59
5 F	52	2,980	414	0.5	14.80
6 F[2]	52	2,881	547	—	—
	$\bar{X}$ = 55.7	$\bar{X}$ = 4,388 ± 625 SEM[3]	$\bar{X}$ = 867.2 ± 248 SEM[3]	$\bar{X}$ = 5.1 ± 1.0 SEM[3]	$\bar{X}$ = 18.2 %

[1]Coefficient of utilization is defined as (increase in weight) × 100/food intake.
[2]Shark no. 6 accidently died after 61 d. Values given are estimates for 95 d.
[3]Standard error of the mean (SEM).

Indoor aquarium, controlled experiment.—Two growth studies on captive lemon sharks were conducted. The first, done for 100 d under controlled conditions with unlimited food, indicated an average daily growth of 0.5 ± 0.1 standard error of mean (SEM) mm/d (Table 3), equivalent to an increase in body length of 19%. The corresponding weight gain was 867.2 ± 248 SEM g or an increase of 19.7%.

Outdoor pond, uncontrolled experiment, and field tagging study.—In the second study, growth measured with unlimited food, in an outdoor pond during the warm summer months, averaged 0.6 ± 0.3 SEM mm/d or an increase of 11% in 89 d. The corresponding weight increased about 32% (Table 4). Field growth, as determined from returns of tagged sharks, was slower than growth of captive sharks. A sample of 16 sharks tagged in 1980 and at large for 7 mo grew at an average rate of 0.2 ± 0.03 SEM mm/d. Another group of 16 sharks tagged in 1967 and 1968 and at large for an average of 3.5 mo, grew at the same rate: 0.2 ± 0.04 SEM mm/d (Table 5).

Monthly catch records.—Several lines of evidence demonstrate that lemon sharks are born in the spring at about 49 cm PCL. Our records show that young lemon sharks are difficult to catch until May (Fig. 6). Through the spring and early summer they become more numerous and are thus easily collected. For example, during the 1980 tagging campaign, we caught < 30 sharks in April, about 50 in May, nearly 475 in June, and almost 500 in July. Even though catch-effort remained relatively high until October, catches fell precipitously to zero by November.

Length-frequency analysis (Fig. 7) of 862 lemon sharks (442 males, 420 females) captured in the summer of 1980 showed a mode at 50 cm PCL. The males averaged slightly smaller (50.8 cm PCL) than the females (51 cm PCL).

To further estimate growth, we compiled catch records on a monthly basis throughout the year. Because lemon sharks are so difficult to find in the winter, we lack data from December and January. However, we were able to obtain at least eight captures for the other months. Shown in Figure 8 are the average size increases throughout the year, peaking in March and April. Thereafter, size ranges fall to the newborn size.

Von Bertalanffy growth model.—We have produced a von Bertalanffy growth curve based on intrauterine growth and maximum size. To this we have added measurements of embryos taken from Springer (1950) and Clark and von Schmidt (1965), as well as our own field data. Holden's (1974) modification of the von Bertalanffy (1960) growth model considerably overestimates growth of the lemon shark (Figs. 9, 10). Only intrauterine growth as determined by measuring embryos throughout the year approximated the curve. Immediately after birth, growth decelerated sharply relative to the theoretical rate. Thus, a 1-yr-old shark 75 cm TL would be predicted by the

Table 4.—Growth of 30 lemon sharks held for 89 d in an outdoor pond (July-September 1980) and fed to satiation daily.

Shark	Precaudal length at start (cm)	Growth in 89 d (mm)	Growth rate (mm/d)	Growth rate (cm/yr)	Weight at start (kg)	Increase in 89 d (kg)	Rate of increase (g/d)	Rate of increase (kg/yr)
1	48.0	65	0.73	27	1.65	0.46	5.1	1.88
2	62.0	105	1.10	40	2.65	2.18	24.0	8.76
3	63.2	68	0.76	28	3.00	4.64	18.4	6.72
4	51.5	71	0.80	29	2.00	0.37	4.1	1.50
5	52.7	65	0.73	27	2.00	0.40	4.5	1.64
6	52.3	52	0.58	21	2.25	0.31	3.5	1.27
7	52.3	49	0.55	20	1.75	0.54	6.1	2.22
8	49.5	49	0.55	20	1.10	0.66	7.4	2.68
9	49.0	55	0.61	22	1.00	1.10	11.8	4.30
10	50.5	59	0.66	24	2.00	0.24	2.7	0.99
11	50.8	50	0.56	20	2.10	0.13	1.5	0.55
12	51.4	48	0.53	19	2.00	0.15	1.6	0.60
13	48.5	45	0.50	18	1.70	0.40	4.3	1.57
14	50.6	59	0.66	24	2.00	0.03	0.4	0.14
15	48.5	50	0.56	20	1.80	0.08	1.0	0.34
16	48.8	52	0.50	18	1.90	0.04	0.4	0.14
17	54.0	55	0.61	22	2.10	0.45	5.1	1.87
18	52.0	37	0.41	15	2.00	0.18	2.0	0.73
19	51.2	63	0.70	26	2.00	0.53	6.0	2.18
20	49.5	65	0.73	27	1.80	0.26	2.9	1.05
21	51.5	55	0.62	23	1.80	0.41	4.6	1.68
22	49.0	65	0.73	27	1.80	0.25	2.8	1.04
23	52.2	43	0.48	18	2.00	0.35	3.9	1.42
24	51.0	52	0.58	21	1.70	0.40	5.1	1.85
25	49.4	51	0.57	21	1.50	0.54	6.0	2.20
26	54.5	65	0.73	27	1.80	1.18	13.2	4.82
27	53.6	49	0.55	20	1.90	0.58	6.6	2.40
28	50.0	87	0.97	35	1.90	0.58	6.5	2.37
29	47.2	70	0.79	29	1.90	0.22	2.5	0.91
30	50.0	36	0.40	15	1.70	0.42	4.7	1.72
	$\bar{X}$ = 51.4 ± 0.6 SEM[1]	$\bar{X}$ = 57.8	$\bar{X}$ = 0.64 ± 0.03 SEM[1]	$\bar{X}$ = 23.3 ± 0.01 SEM[1]	$\bar{X}$ = 1.87	$\bar{X}$ = 0.60	$\bar{X}$ = 5.61 ± 1.8 SEM[1]	$\bar{X}$ = 20.5 ± 0.68 SEM[1]

[1]Standard error of the mean (SEM).

Table 5.—Growth of the lemon shark based on tag-recapture data. Upper group is from sharks marked and released in 1980; lower group was marked and released in 1967 by Starck (text footnote 3).

Precaudal length when tagged (cm)	Time at large (d)	Increase in size (mm)	Growth (mm/d)	Growth (cm/yr)
41	219	30	0.14	5.1
70	362	80	0.22	8.0
55	235	40	0.17	6.2
47	532	134	0.25	9.1
51	10	0	0.00	0.0
48	38	8	0.21	7.7
50	60	31	0.52	19.0
53	132	29	0.22	8.0
48	167	40	0.24	8.8
50	290	68	0.23	8.4
51	193	64	0.33	12.1
47	327	98	0.29	10.6
48	38	3	0.08	2.9
52	53	3	0.06	2.2
49	480	142	0.30	11.0
59	351	22	0.06	2.2
$\bar{X}$ = 51.2 ± 1.60 SEM[1]	$\bar{X}$ = 218	$\bar{X}$ = 49.5	$\bar{X}$ = 0.21 ± 0.03 SEM[1]	$\bar{X}$ = 7.7 ± 1.10 SEM[1]
57	124	10	0.08	2.9
53	147	35	0.24	8.8
53	120	25	0.21	7.7
66	365	25	0.07	2.6
59	124	20	0.16	5.8
53	207	10	0.05	1.8
56	207	20	0.10	3.7
47	13	5	0.38	13.9
50	12	5	0.42	15.3
57	192	40	0.21	7.7
48	10	5	0.50	18.3
48	132	35	0.27	9.9
56	120	35	0.29	10.6
50	56	5	0.09	3.3
50	164	25	0.15	5.5
46	62	40	0.65	23.7
$\bar{X}$ = 53.0 ± 1.30 SEM[1]	$\bar{X}$ = 128	$\bar{X}$ = 21.3	$\bar{X}$ = 0.24 ± 0.4 SEM[1]	$\bar{X}$ = 8.8 ± 1.50 SEM[1]

[1]Standard error of the mean (SEM).

model to be 110 cm TL, a 46% overestimation. Actually, our tag returns suggest that a 110 cm specimen is in year 6.

By assuming formation of three growth bands on the centrum face each year, and plotting the vertebral results, the theoretical model still overestimates growth. For example, we estimate a 223 cm TL shark to be in its 9th year since its centrum face possesses 28 growth bands. Yet the Holden (1974) model predicts that a 9-yr-old lemon shark will be 310 cm TL, a 39% overestimation. We await further biological materials, especially from larger specimens, to produce a reliable von Bertalanffy growth model (or alternative model) for this species.

DISCUSSION

Results presented herein are a prelude to a more complete study on age and growth in the lemon shark. Yet, the data represent the first validated ageing of a tropical shark. One advantage of this investigation was that we were able to assess age and growth by several independent methods. This arose because of the basic biology and life history of the lemon shark. For example, lemon sharks are numerous, localized, viviparous, reproductively seasonal, and live well in captivity.

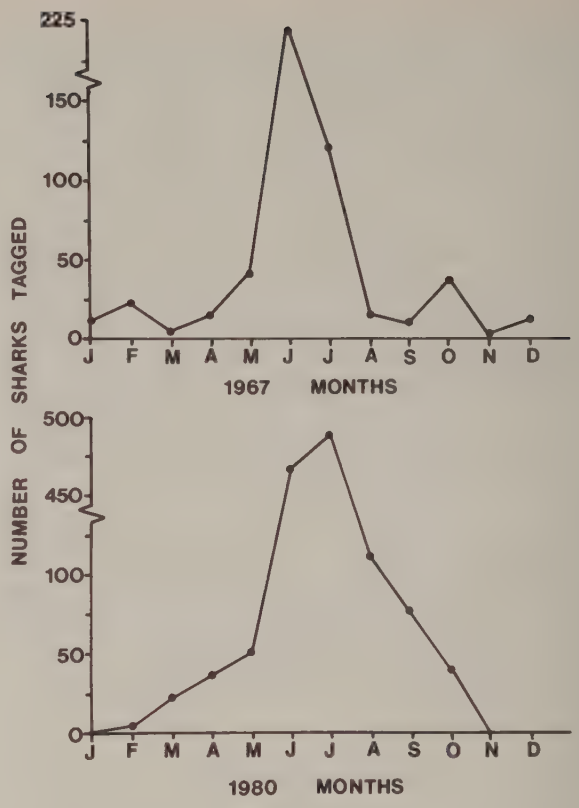

Figure 6.—Number of lemon sharks tagged in 1967 (see text footnote 1) and 1980.

Thus, we were able to gather laboratory and field information on size-at-birth, growth rates, length-frequency, tetracycline uptake, and formation of growth zones on vertebral centra.

Vertebral Centra

Interpretation of growth zones on vertebral centra provides the most widely reported data on the age of elasmobranchs (Hoenig 1979). Vertebral centra of several carcharhinid species possess easily distinguished concentric growth patterns (Haskell 1949; Stevens 1975), and so we expected the lemon shark to conform. We tried several methods to enhance growth bands including xylene clearing (Daiber 1960), hematoxylin and eosin (Ishiyama 1951), and silver nitrate staining (Stevens 1975). However, the clearest differentiation was obtained with alizarin red S (La Marca 1966). We intend to reexamine the silver nitrate stain by following the procedure given in Cailliet et al. (1983), since a demonstration of their technique on lemon shark centra (at the workshop) brought about very clear growth bands in a matter of minutes.

The problem of subjective error (i.e., precision; see Glossary) is inherent in counting growth zones. This problem is exacerbated with older specimens, especially at the edge of the centrum where bands are compressed and more difficult to count and measure. However, Jones and Geen (1977) have established an objective method of counting growth bands. They produced thin cross sections of centra from the spiny dogfish,

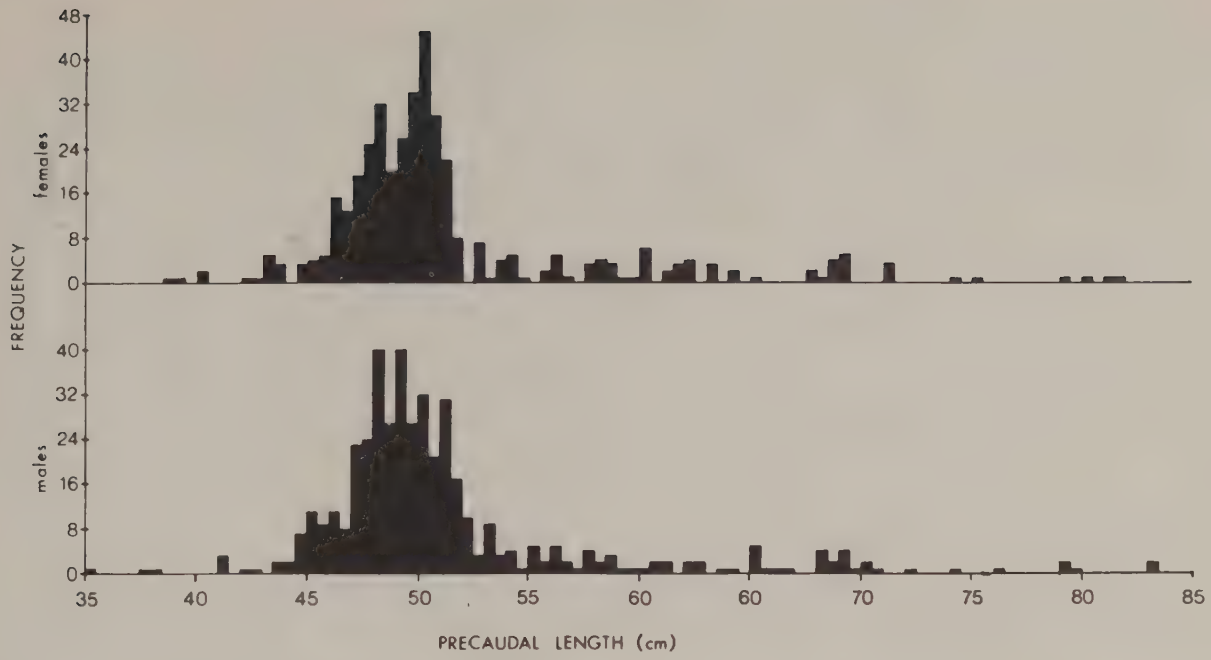

Figure 7. – Length-frequency histogram of 862 lemon sharks tagged and released in the summer of 1980. The upper histogram represents 420 females and the median PCL was 49.5 cm; the mean was 51.0 cm PCL. The lower histogram represents 442 males and the median was 49.25 cm PCL; the mean was 50.8 cm PCL.

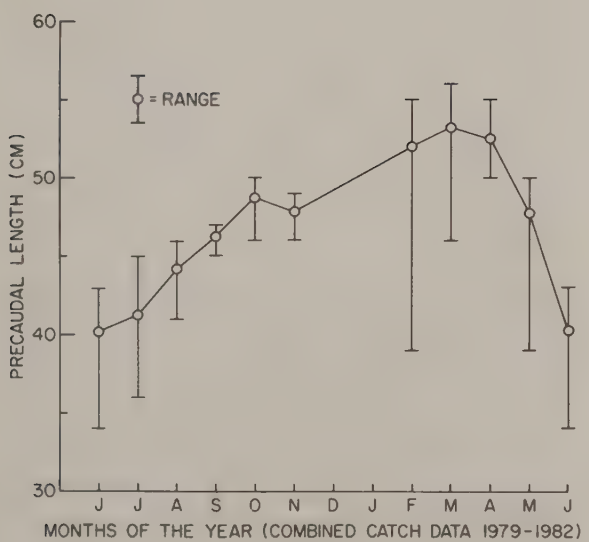

Figure 8.—Annual increase in precaudal length of young-of-the-year lemon sharks. All sharks captured in a particular month over a period of 3.5 yr were ranked by size and the average precaudal length of the 10 smallest sharks in the monthly samples was calculated. Vertical lines are the range of sizes in the subsample. The smallest lemon sharks grow about 15 cm/yr and parturition begins in May.

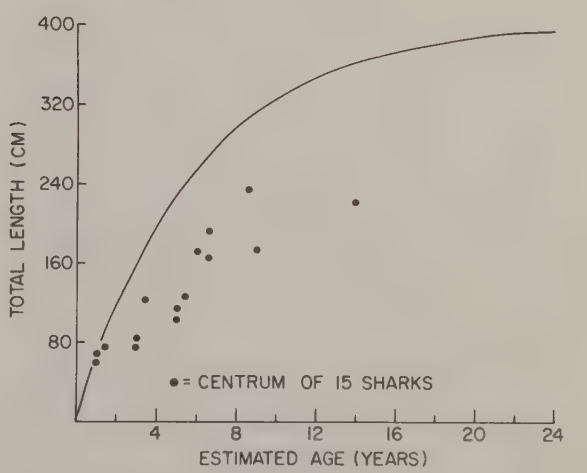

Figure 9.—Relationship between the theoretical von Bertalanffy growth model (smooth curve) and estimated age-at-length of lemon sharks (solid circles). Age was estimated by assuming two growth bands are produced annually, thus the number of bands on an alizarin-stained centrum from each of 15 sharks was divided by two and plotted as estimated age. The von Bertalanffy curve was generated by computer from parameters given in Equation (1) of the text.

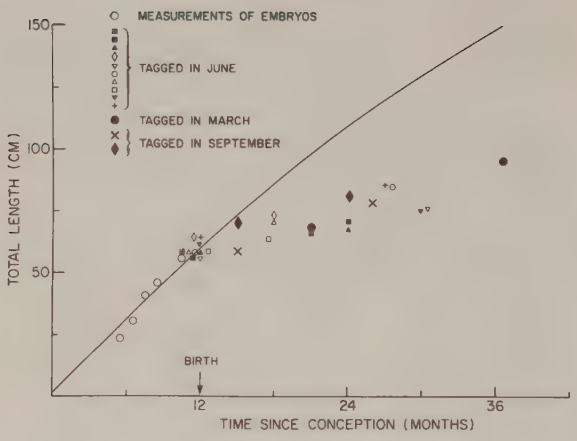

Figure 10.—Relationship between the theoretical von Bertalanffy growth model (continuous curve) and growth of 10 tagged lemon sharks (shown as 10 different symbols). The von Bertalanffy curve was generated by computer from parameters given in Equation (1) of the text. Date and size of 10 newborn lemon sharks, tagged and released in June (assumed birth month), and recaptured later, are plotted along with two September and one March release-recaptures. Average measurements of late embryos (open circle with dot) from November through April (Springer 1950; Clark and von Schmidt 1965) are also shown.

Squalus acanthias, and demonstrated periodic variations in the concentration of calcium and phosphorus by X-ray spectroscopy. The variations generally corresponded to growth bands, and resolution of calcium peaks, particularly at the edge of the centrum, provided objective counts of the total number of growth bands. To evaluate subjective error and obtain an accurate count of the number of growth bands for age estimation, we intend to analyze samples of lemon shark centra by X-ray spectroscopy.

Subjective error notwithstanding, the vertebral centrum appears to be an effective structure for ageing lemon sharks. Growth of the centrum is linear and this agrees with reports on a sphyrnid and two carcharhinid sharks (Hoenig 1979), the blue shark (Stevens 1975), and the Japanese dogfish (Tanaka and Mizue 1979). Similar findings are reported in the shark papers from this workshop. In contrast, centrum growth in the black skate is exponential (Ishiyama 1951). The combination of linear growth and periodic growth marks justifies back calculation of the previous growth history of a vertebra and should allow us to calculate the length of a shark for each estimated age represented by a growth band (Bagenal and Tesch 1978; Smith 1983). Such back calculations will be attempted when a representative sample of centra from all size ranges of lemon sharks becomes available.

The major finding from stained vertebrae is that lemon sharks form periodic growth marks on their vertebral centra and the number of these increases with size. Specimens that we identify as young-of-the-year have from one to three growth bands. We counted up to 28 bands in nearly mature specimens of 250 cm TL. Thus, we have established that the concentric bands in the centra represent a periodic growth mark that begins at an early age. However, if we are to reliably assign an age to these specimens, we must specify a regular time scale or interval between formation for these marks (i.e., we must validate the growth marks).

Tetracycline Validation

Bagenal and Tesch (1978) listed several techniques for validation, including comparison of growth checks with Petersen data as well as marking, releasing, and rearing experiments (Brothers 1983). Still, these validations depend to some extent on circumstantial evidence. One of the most direct validation methods is by treatment with tetracycline or other chemicals that form permanent marks on the hardpart being examined (Weber and Ridgway 1962). Data from our tetracycline tag returns to date clearly demonstrate that detailed tetracycline validation of growth bands will become a reality for this species. In this regard, of the several tetracycline experiments with elasmobranchs, sharks appear to be somewhat better subjects than the batoids. Holden and Vince (1973) and Martin[4] reported broadly diffuse fluorescent rings in batoids after tetracycline treatment, which suggests a relatively slow process of incorporation. In contrast, the lemon shark, leopard shark, *Triakis semifasciata* (Smith[5]), and spiny dogfish, *S. acanthias* (Tucker[6]; MacLellan[7]), all formed discrete fluorescent bands. In the lemon and leopard sharks, discrete banding is correlated with relatively rapid incorporation of tetracycline into hardparts: About 30 d is required for the lemon shark and 22 d for the leopard shark (Smith footnote 5). In contrast, goldfish require up to 60 d for inclusion in hardparts (Kobayashi et al. 1964), while tetracycline binds almost immediately in salmon (Weber and Ridgway 1967) and man (Frost 1969).

The variability of ring formation with constant dosage found in this study agrees with the findings of Kobayashi et al. (1964), Weber and Ridgeway (1967), Holden and Vince (1973), and Smith (footnote 5) who worked on other species. However, controlled experiments are underway to determine the critical range of both ingested and injected tetracycline necessary for ring formation in the centrum of the lemon shark.

A major finding of the tetracycline experiment was the demonstration that the first growth band is elaborated several weeks after birth. Thereafter, for at least the first 30 mo, a fall band and a late winter band appear to be formed annually. If this can be confirmed in older specimens, we will be able to accurately age any lemon shark by counting growth bands. At present, we have tetracycline-treated vertebrae from nearly 100 lemon sharks up to 3 yr of age and substantiation of these preliminary data is underway.

Growth Rate

Growth of the lemon shark has been evaluated several times in the past (Springer 1939, 1950; Clark and von Schmidt 1965; Moss 1972). An average growth rate of 90 cm/yr calculated from these studies suggests that this species is a rapidly growing tropical shark (Stevens 1975) maturing in < 3 yr (Table 6). However, our findings both from growth under controlled conditions and from tag returns stand in strong contrast to the

[4]L. K. Martin, Fishery Biologist, Moss Landing Marine Laboratory, Moss Landing, CA 95039, pers. commun. 1981.

[5]S. Smith, Fishery Biologist, Southwest Fisheries Center Tiburon Laboratory, National Marine Fisheries Service, NOAA, 4150 Paradise Drive, Tiburon, CA 94920, pers. commun. 1982.

[6]R. Tucker, Fishery Biologist, Fisheries Laboratory, Lowestoft, Suffolk NR3 0HT, United Kingdom, pers. commun. 1982.

[7]S. MacLellan, Fishery Biologist, Pacific Biological Station, Nanimo, B. C. Canada, V9R 5K6, pers. commun. 1982.

Table 6.—Growth and estimated age at maturity[1] for lemon sharks from various sources.

Growth rate		Estimated age at maturity[1]		
mm/d	cm/yr	(yr)	Type of observation	Source
0.22	8	23.7	Tagging returns, average 294 d at liberty. $n = 15$	Present study
0.24	9	21.6	Tagging returns, average 128 d at liberty. $n = 16$	Starck (text footnote 3)
0.53	20	9.5	Growth under controlled conditions, 100 d, $n = 6$	Longval et al. (1982)
0.63	23	8.2	Growth in a pond, July through September, 100 d, $n = 30$	Present study
0.86	31	6.1	Growth under controlled conditions, Sea World, 480 d, $n = 3$	Keyes[2]
2.01	73	—	Average size of embryos removed from gravid females, December through April	Springer (1950)
2.03	74	2.6	170 cm female kept in pond, April to September, $n = 1$	Clark and von Schmidt (1965)
2.26	78	2.3	Growth under controlled conditions, New England Aquarium, final length estimated. 290 d. $n = 1$	Casey[3]
2.50	91	2.1	Estimated growth from tooth replacement rates on captive sharks, $n = 4$	Moss (1972)
2.83	103	1.5	Observed young lemons in an inlet, possibly born there. Caught 2 on "birthday" and 2 others 40 d later. $n = 2$	Springer (1938)

[1]Assumes linear growth rate and maturity at 250 cm TL.

[2]R. M. Keyes, Curator of Fishes, Sea World, 1720 South Shores Road, San Diego, CA 92109, pers. commun. 1982.

[3]J. G. Casey, Fishery Biologist, Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA, Narragansett, RI 02820, pers. commun. 1982.

earlier findings. We estimate that growth of young lemon sharks does not exceed 25 cm/yr and probably lies between 10 and 20 cm/yr. One possible reason for this discrepancy may be that most of the observations listed in Table 6 were made on captive sharks. It has long been known that sharks can grow rapidly in captivity. For example, Wass (1971) reported that the grey reef shark, *Carcharhinus amblyrhynchos*, grew 10 times faster in captivity than conspecifics grew in the field. Lemon sharks may grow up to 9 times faster than tagged conspecifics at liberty (Table 6). Thus, it seems that charcharhinid sharks may have an inherent capacity to take advantage of favorable conditions by increasing growth during periods of abundant food. Such a strategy would have high survival value.

Taking all our evidence into account, the tag returns probably provide the best estimates of age and growth because they represent a relatively large sample from two temporally discontinuous populations. Tagging, of course, could affect growth so we investigated this possibility (Gruber 1982) by placing 36 sharks in a pond and separating them into five experimental groups with various tagging combinations and a control group (no marks). The control group grew some 10% faster but the difference was not statistically significant (Gruber 1982). Thus, tagging per se does not greatly affect the growth rate when food is unlimited. At a growth rate of 15 cm/yr, maturity is not attained until a dozen or more years. This means that the lemon shark is a slow-growing, late-maturing species similar to the sandbar shark, *C. plumbeus* (Casey et al. 1983).

Our catch records (Figs. 6, 7) and incidental measurements of size frequency by month establish that numerous newborn lemon sharks of about 49 cm PCL appear in shallow waters off the Florida Keys in spring and early summer. Bigelow and Schroeder (1948), Clark and von Schmidt (1965), and Springer (1939, 1950) made similar observations. Their studies of embryos, young, and reproductively active adults confirmed that parturition and probably copulation are restricted to a period between May and July. Indirect evidence indicated a gestation period of 1 yr, and since only 50% of mature females they examined were pregnant, Clark and von Schmidt (1965) suggested that females may reproduce every other year. Lemon sharks give birth to an average of 11 pups. Thus, they can reasonably be described as reproductively seasonal, with low fecundity, slow growth, and late maturity. Similar conclusions have been arrived at independently by Wass (1971), Holden (1974), Cailliet et al. (1983), and Casey et al. (1983), for other carcharhinid species. The potentially disastrous consequences of intense fishing pressure on sharks with this type of life history have been treated by others at this workshop and in Holden (1974, 1977). We feel that the lemon shark is a prime candidate to be added to this list of susceptible elasmobranchs and note, with concern, the existence of an unannounced gill net fishery on the Florida Keys nursery grounds for this species.

Results of this study appear to clarify several aspects of the life cycle of the lemon shark in this geographical area. Life begins in the late spring and early summer with sharks of about 49 cm PCL born on nursery grounds in the shallow waters around the Florida Keys. Springer (1950) reported size at birth of 66 cm TL (53 cm PCL) but we would consider this a very unusual case, at least for the Florida Keys population. Growth during the first year proceeds at a rate of 0.2 to 0.4 mm/d and a yearling will measure about 60 cm PCL. The 45th centrum

will have grown in diameter from 5.0 mm at birth to 6.9 mm at 1 yr and will have at least 2 and possibly 3 growth bands, one formed in the fall 3 mo after birth and a second formed in late winter. Growth continues at about 0.3 mm/d so that a 2+ yr-old shark is about 71 cm PCL. Its vertebrae will have grown in direct proportion to its change in length and its 45th centrum will bear 5 to 6 bands. A shark in its third year will have reached perhaps 79 cm PCL and bear 7 to 9 growth bands on its centrum.

Lemon sharks become sexually active at approximately 250 cm TL (Springer 1950; Clark and von Schmidt 1965). Assuming the unlikely case of continuous, undecelerated growth of 15 cm/yr, more than 12 yr are required for the lemon shark to reach sexual maturity and over 20 yr to attain maximum size. More likely, growth slows considerably as the shark ages. Many reports of negative or no growth of tag returns on mature sharks have led to the conclusion that shark growth reaches an asymptote and that sharks may live many years thereafter. Perhaps the most striking case is that of a mature school shark, *Galeorhinus australis*, internally tagged by Olsen in 1951 and recaptured in 1976. This shark had grown < 1 cm in the intervening 25 yr (Anonymous 1976). Wass (1971) and Casey et al. (1983) reported that sandbar sharks at liberty several years did not grow. Thus, a mature lemon shark is probably much older than 12 yr.

Our final point is that, like the sandbar shark (Casey et al. 1983), the lemon shark moves offshore as an adult. If food availability were greater, growth rate could increase at that time. Thus, as a consequence of change in habitat, the growth model for this species could be ogival rather than asymptotic as required by the von Bertalanffy model.

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Glossary

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CHARLES A. WILSON, Chairperson
EDWARD B. BROTHERS
JOHN M. CASSELMAN
C. LAVETTE SMITH
ALEX WILD

All terms in this glossary are defined as they relate to skeletal hardparts (vertebrae, spines), otoliths, and scales.

Annual mark (year marks)—Structural features that correlate with a yearly event.

Annulus (annular mark)—A concentric zone, band, or mark that is either a ridge or valley or translucent or opaque. A unit passage of time (i.e., 1 yr) is not inherently implied, unless specified. However, this term has been traditionally used to designate year marks.

Band (rings, marks, zones)—Terms used as auxilliary descriptive words.

Check—An abrupt discontinuity in a zone or band.

Circulus—The concentric bony ridge on fish scales.

Cohort—An age class; a group of fish nearly the same age (difficult to use if the fish being considered is a continuous spawner).

Core—The concentric area of non-incremental growth (reference to otoliths) surrounding the primordium or primordia (see primordium).

Focus—The hypothetical or real origin of the skeletal structure being examined. Traditionally refers to scales but may be used in a general sense for fin spines, fin rays, vertebrae, or otoliths.

Growth increment—In the most general sense, a defined quantity of growth. A general reference to material that exhibits a repetitive lamellar structure corresponding to a unit passage of time. The dimensions, chemical composition, and period of formation will vary widely depending upon the skeletal hardpart involved.

Marginal increment—The region beyond the last identifiable mark at the margin of a skeletal hardpart. Ideally, this area should be expressed in relative terms, i.e., the fraction or proportion of the last complete growth increment.

Opaque—A zone that inhibits the passage of light.
Transmitted light: Opaque zone appears dark; translucent zone appears bright.
Reflected light: Opaque zone appears bright; translucent zone appears dark.

Primordium—A self-contained zone that represents the point (or points) of the original growth of an otolith.

Radius—A defined measure from a focus to a specified point (mathematically incorrect).

Soft ray—1) Opened base.
2) Branched distally.
3) Always segmented.
4) Distal radial within open base.
5) Right and left halves either separate or with a mid-sagittal suture.

Spinous ray (fin spine, spine)—1) Closed base.
2) Unbranched distally.
3) Unsegmented.
4) Distal radial always outside spine.
5) No separation into right halves.

Translucent—A zone that allows the passage of light.

Validation—The confirmation of the temporal meaning of a growth increment. Analogous to determining accuracy of age determination; used in reference to true (absolute) age.

Verification—The confirmation of a numerical interpretation. Analogous to determining precision (reproducibility) of age determination; used in reference to the precision of estimated (presumed) age.

Sections of structures under examination are described under the following criteria based on the position of the skeletal hardpart relative to the organism being examined (Fig. 1).

A. Transverse (cross) section.
B. Longitudinal—horizontal = frontal
vertical = sagittal
mid—a cut through the center.
para—a cut that is off center.

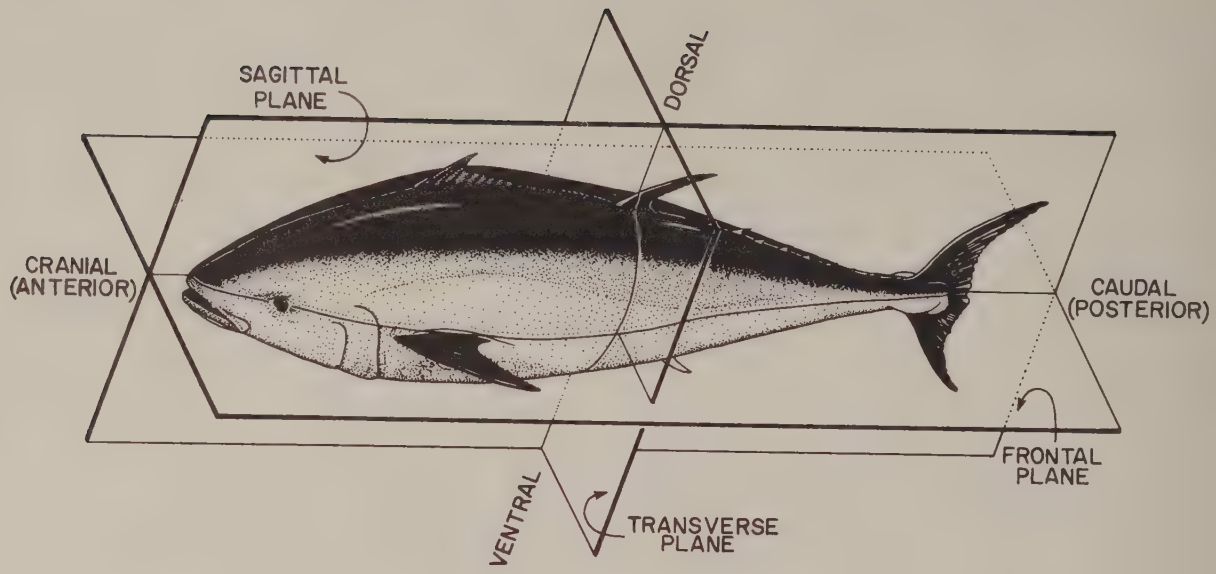

Figure 1.—Axes and planes of an oceanic pelagic fish (tuna).

WORKSHOP PARTICIPANTS

ANTOINE, LOIC M.
Centre Oceanologique de Bretagne
29-N Plouzane
Boite Postale 337
Brest Cedex 29273
FRANCE

ASHE, PAULA
South Carolina Marine Resources Research Institute
Charleston, SC 29412

BARGER, LYMAN E.
Panama City Laboratory, SEFC
3500 Delwood Beach Road
Panama City, FL 32407

BARTOO, NORM W.
La Jolla Laboratory, SWFC
P.O. Box 271
La Jolla, CA 92038

BEARDSLEY, GRANT L.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

BERKELEY, STEVEN A.
RSMAS, University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149

BERRY, FRED H.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

BRANSTETTER, STEVEN
Department of Wildlife & Fisheries
Texas A & M University
College Station, TX 77843

BROTHERS, EDWARD B.
Section of Ecology & Systematics
Cornell University, Corson Hall
Ithaca, NY 14853-0239

CAILLIET, GREGOR M.
Moss Landing Marine Laboratory
P.O. Box 223
Moss Landing, CA 95039

CASEY, JOHN G.
Narragansett Lab, NEFC
RR7-A, Box 552-A
South Ferry Road
Narragansett, RI 02882

CASSELMAN, JOHN M.
Ministry of Natural Resources
Research Scientist, Fisheries Branch
P.O. Box 50
Maple, Ontario
CANADA L0J 1E0

CAYRÉ, PATRICE M.
Republique du Senegal
Institut Senegalais de Recherches Agricoles
Centre of Recherches Oceanographiques de Dakar-Thigroye
B.P. 2241 - Dakar
REPUBLIQUE OF SENEGAL

COLVOCORESSES, JAMES A.
Virginia Institute of Marine Science
Gloucester Point, VA 23062

COMPEÁN-JIMENEZ, GUILLERMO
Centro de Investigaciones Pesqueras, I.N.P.
Apartado Postal 976
Ensenada, Baja California
MEXICO

CONSER, RAMON J.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

CROW, MICHAEL E.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

DEAN, JOHN M.
Belle W. Baruch Institute for Marine Biology & Coastal Research
University of South Carolina
Columbia, SC 29208

FABLE, WILLIAM A., JR.
Panama City Laboratory, SEFC
3500 Delwood Beach Road
Panama City, FL 32407

FARBER, MARK I.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

FOREMAN, TERRY J.
Inter-American Tropical Tuna Commission
c/o Scripps Institute of Oceanography
La Jolla, CA 92034

FORMAN, STEVEN R.
RSMAS, University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149

FOX, WILLIAM W., JR.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

GONZÁLEZ-GARCÉS, ALBERTO
Oceanografo-Biologo
Intituto Espanol De Oceanografia
Apartado 130
La Coruna - SPAIN

GOURLEY, JOHN E.
Virginia Institute of Marine Science
Gloucester Point, VA 23062

GRALL, CHARLENE
RSMAS, University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149

GRUBER, SAMUEL H.
RSMAS, University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149

HALES, STAN
Grice Marine Laboratory
Charleston, SC 29412

HEDGEPETH, MARION Y.
Florida Department of Natural Resources
West Palm Beach Field Station
727 Belvedere Road
West Palm Beach, FL 33405

HOUDE, EDWARD D.
Chesapeake Biological Lab
P.O. Box 39
Solomons, MD 20688

HURLEY, PETER C. F.
Government of Canada, Fisheries & Oceans
Marine Fish Division
P.O. 1006
Dartmouth, Nova Scotia
CANADA B2Y 4A2

JAVECH, JACK C.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

JOHNSON, ALLYN G.
Panama City Laboratory, SEFC
3500 Delwood Beach Road
Panama City, FL 32407

JOHNSON, G. DAVID
South Carolina Marine Resources Research Institute
Charleston, SC 29412

KUSHER, DAVID
University of Georgia
Department of Zoology
Athens, GA 30603

LAWLER, EDWARD F.
Virginia Institute of Marine Science
Gloucester Point, VA 23062

LEE, DENNIS W.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

LOPEZ, A. MONTY
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

MacLELLAN, SHAYNE E.
Government of Canada, Fisheries & Oceans
Pacific Biological Station
Nanaimo, British Columbia
CANADA V9R 5K6

MAJKOWSKI, JACEK
CSIRO Marine Laboratories
Division of Fisheries
P.O. Box 21
Cronulla, NSW 2230
AUSTRALIA

MAMMACK, MARTA
Virginia Institute of Marine Science
Gloucester Point, VA 23062

MANN, WALTER C.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

MARCHETTE, DON
South Carolina Marine Resources
Box 12559
Charleston, SC 29412

MARTIN, LINDA K.
Moss Landing Marine Laboratory
P.O. Box 223
Moss Landing, CA 95039

McGOWAN, MICHAEL F.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

MENDOZA, JEREMY J.
Centre Oceanologique de Bretagne
29-N Plouzane
Boite Postale 337
Brest Cedex 29273
FRANCE

NICHOLS, SCOTT
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

PALKO, BARBARA J.
Panama City Laboratory, SEFC
3500 Delwood Beach Road
Panama City, FL 32407

POWERS, JOSEPH E.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

PRATT, HAROLD L.
Narragansett Lab, NEFC
RR7-A, Box 552-A
South Ferry Road
Narragansett, RI 02882

PRINCE, ERIC D.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

RADTKE, RICHARD L.
University of Hawaii at Manoa
Hawaii Institute of Marine Biology
P.O. Box 1346
Coconut Island
Kaneohe, HI 96744

RICHARDS, WILLIAM J.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

ROFFER, MITCHELL A.
RSMAS, University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149

SCHATMAN, RONALD
2300 NE. 172nd Street
North Miami Beach, FL 33160

SCHWARTZ, FRANK J.
UNC Institute of Marine Science
3407 Arendell Street
Morehead City, NC 28557

SMITH, C. LAVETT
American Museum of Natural History
Central Park West at 79th Street
New York, NY 10024

SMITH, TED
South Carolina Marine Resources
Box 12559
Charleston, SC 29412

STILLWELL, CHARLES E.
Narragansett Lab, NEFC
RR7-A, Box 552-A
South Ferry Road
Narragansett, RI 02882

STOUT, ROBERT G.
RSMAS, University of Miami
4600 Rickenbacker Causeway
Miami, FL 33149

THORSON, THOMAS B.
School of Life Science
University of Nebraska - Lincoln
Lincoln, NE 68588

WELDEN, BRUCE A.
Moss Landing Marine Laboratory
P.O. Box 223
Moss Landing, CA 95039

WILD, ALEX
Inter-American Tropical Tuna Commission
c/o Scripps Institute of Oceanography
La Jolla, CA 92037

WILSON, CHARLES A.
Belle W. Baruch Institute for Marine Biology & Coastal Research
University of South Carolina
Columbia, SC 29208

WILSON, TERESA A.
Southeast Fisheries Center
75 Virginia Beach Drive
Miami, FL 33149

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